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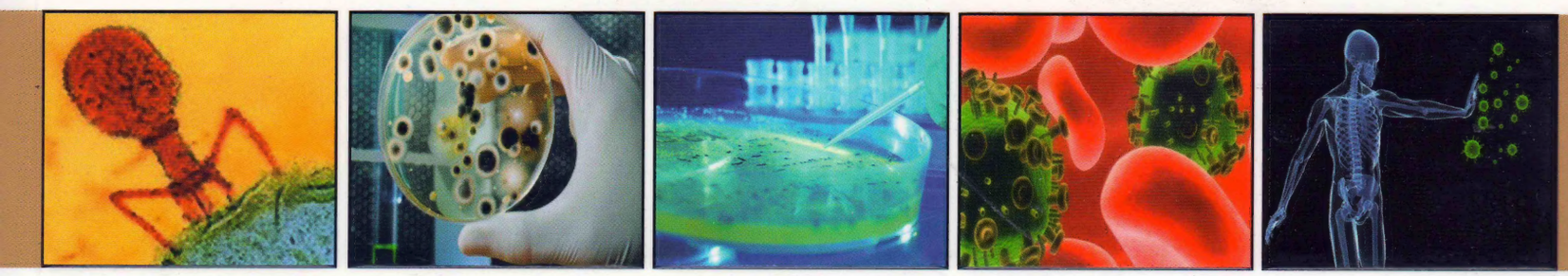

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Self-Assessment and Review of

MICROBIOLOGY AND IMMUNOLOGY

12th Edition

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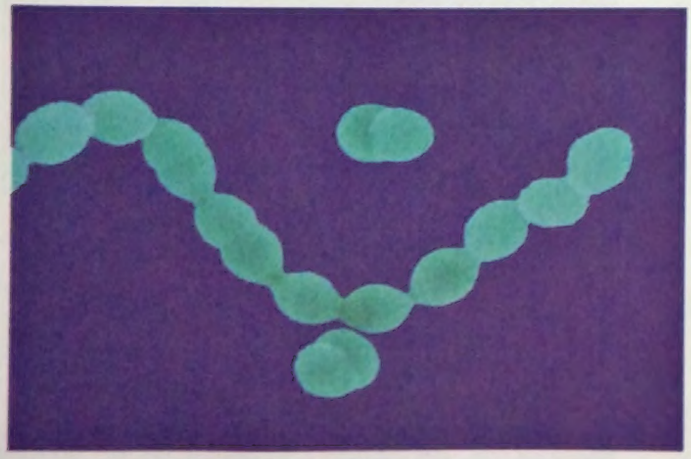
IMAGE BASED QUESTIONS

IMAGE BASED QUESTIONS

1. **A dutch trades man whose hobby was to make lenses. His image is shown below. Identify the great scientist:**

a. Robert Koch
b. A.V Leeuwenhoek
c. Louis Pasteur
d. Edward Jenner

2. Which type of bacterial morphology is shown below:



a. Pneumococcus
b. Streptococcus
c. Staphylococci
d. Meningococci

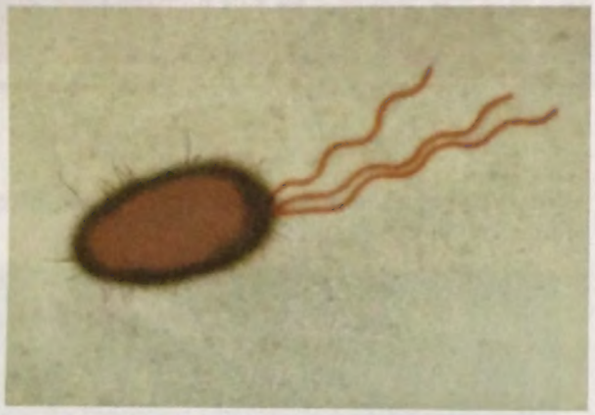
3. **What type of genetic exchange is being exhibited by the *E. coli* in the picture below?**

a. Conjugation
b. Transduction
c. Transformation
d. Mutation

4. **What group of organisms is represented by the image below?**

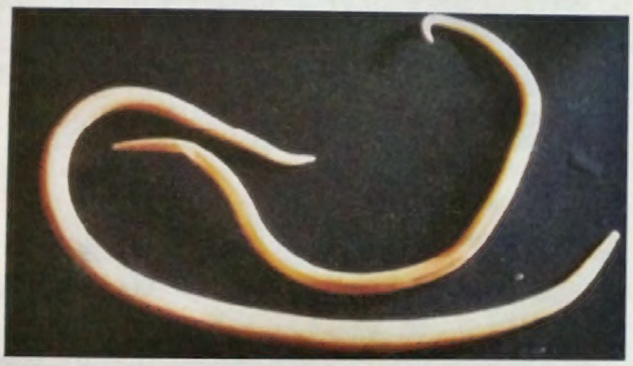
a. Fungi
b. Bacteria
c. Virus
d. Filaria

5. **What flagella arrangement is exhibited in the image below?**



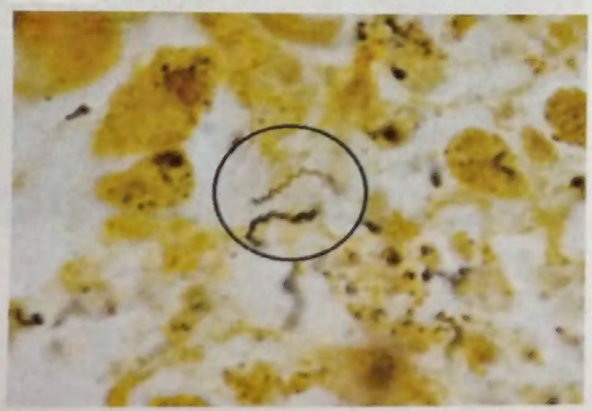
a. Peritrichous
b. Lophotrichous
c. Monotrichous
d. Amphitrichous

6. **What classification group of parasite is shown in picture?**



a. Protozoa
b. Nematoda
c. Cestode
d. Trematoda

7. **The microorganism circled in the image below is the causative agent of a disease that is transmitted sexually. Identify the organism**



a. Gonococci
b. Treponema Pallidum
c. Hemophilus ducreyi
d. Calymmobacter granulomatis

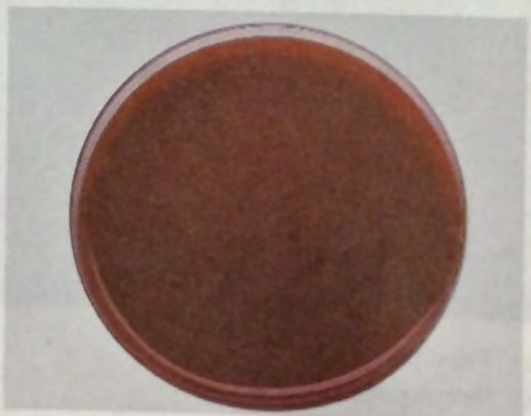
8. **The symbol shown in the image below represent:**

a. Radiation hazard
b. Cytotoxic hazards
c. Bio hazard
d. Magnetic field inside

9. **Identify the instrument:**

a. Tuberculin syringe
b. Insulin syringe
c. 2ml syringe
d. Micro syringe

10. **Identify the material shown below in petridish:**



a. Choclate Agar
b. Blood Agar
c. LJ Medium
d. VP medium

EXPLANATIONS

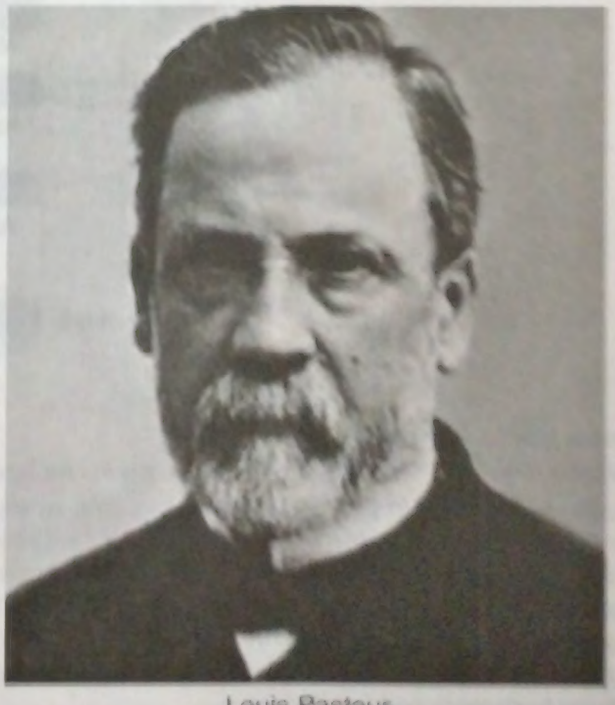
1. Answer is b. A.V Leeuwenhoek

IMAGES OF SOME FAMOUS SCIENTISTS

Robert koch

A.V Leeuwenhoek

Edward Jenner

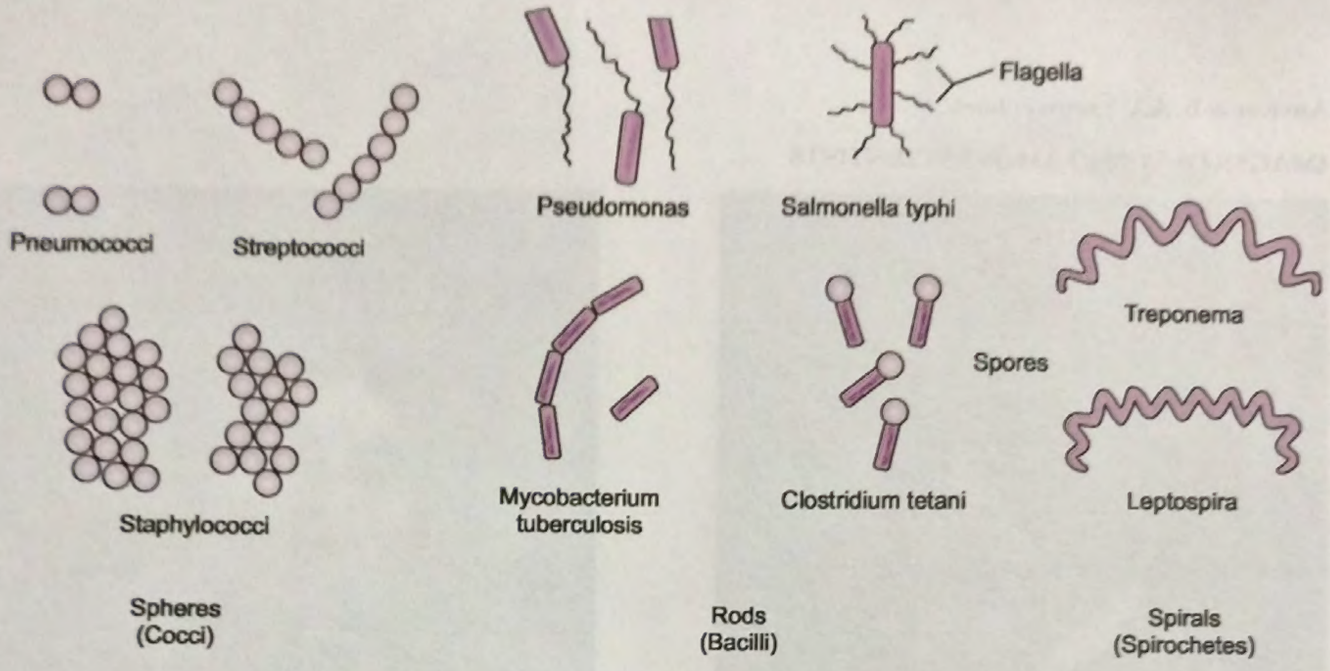


Louis Pasteur

2. **Answer is b i.e Streptococcus**

Gram (+)ve cocci arranged in linear cluster can be none other than Streptococci. Staphylococci divide is three planes to form grape like clusters.

Growth pattern of some important bacteria



3. **Answer is a i.e Conjugation**

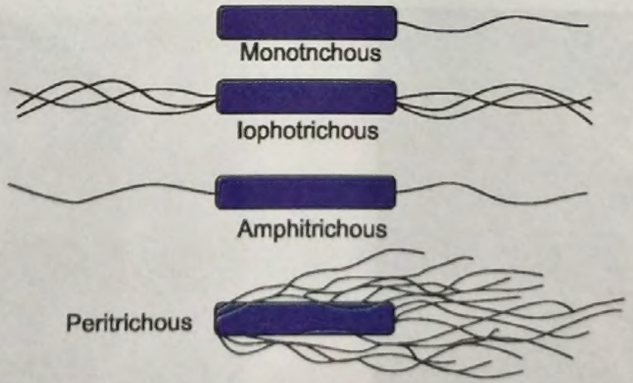
Here one can see the presence of sex pilli (red in image)

4. **Answer is a i.e. Fungi**

Note the bluish spheres these are spores

5. **Answer is b i.e. Lophotrichous**

Types of flagella



6. **Answer is b i.e Nematoda**

A parasite with long thin ungesmented tubular body can be none other than nematode. (See image below)

Nematode (roundworms): They have long thin unsegmented tube-like bodies with anterior mouths and longitudinal digestive tracts. They have a fluid-filled internal body cavity (pseudocoelum) which acts as a hydrostatic skeleton providing rigidity (so-called '***tubes under pressure***'). Adult worms form separate sexes with well-developed reproductive systems.

Cestodes (tapeworms): They have long flat ribbon-like bodies with a single anterior holdfast organ (scolex) and numerous segments. They do not have a gut and all nutrients are taken up through the tegument. They do not have a body cavity (acoelomate) and are flattened to facilitate perfusion to all tissues. All tapeworms are hermaphroditic and each segment contains both male and female organs.

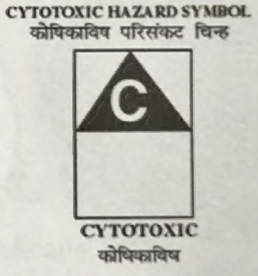
Trematodes (flukes): They have small flat leaf-like bodies with oral and ventral suckers and a blind sac-like gut. They do not have a body cavity (acoelomate) and are dorsoventrally flattened with bilateral symmetry. Most species are hermaphroditic (individuals with male and female reproductive systems) although some blood flukes form separate male and female adults

Nematode Cestode Trematode

7. **Answer is b i.e. Treponema Pallidum**
   Refer answer no. 2 for image of Treponema

8. **Asnwer is c i.e Bio hazard**
   Some Important medical symbols

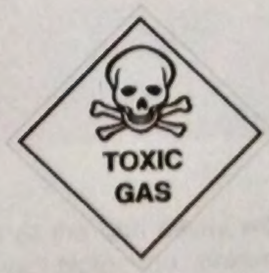
Biohazard



Cytotoxic Hazard

Radiation Hazard

Magnetic field symbol

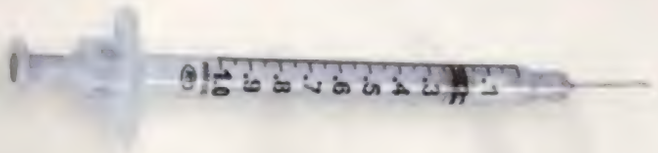


Toxis Gas

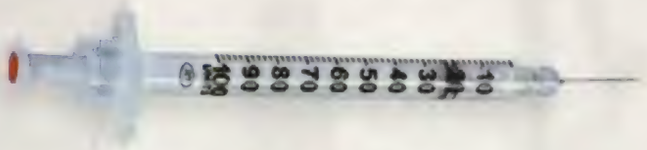
Toxis Chemicals

9. **Answer is a i.e. Tuberculin syringe**

Tuberculin syringe has calibrations from 0.1 to 1.0 where as insulin syringe has calibrations either up to 40 or up to 100 which represent 40 IU and 100 IU respectively. See figure below



Tuberculin syringe



Insulin syringe

10. **Answer is b i.e. Blood Agar**

Image plates of some important culture mediums

Choclate Agar

LJ Media

OTHER INFORMATIVE IMAGES

Figure: The distribution and comparison of the rash of rubeola, rubella, scarlet fever, and chickenpox, which can be helpful in determining the diagnosis of these childhood exanthems.

Figure: A photograph of an infection of the oral cavity with thrush caused by *Candida albicans*. Note the creamy appearance of the pseudomembrane and the erythematous appearance of the mucosa.

Figure: Gram stain of *Neisseria meningitidis*. Notice the gram-negative diplococci (black arrows) joined together on their longer side.

Figure: A child with a deformity of the right lower leg due to poliomyelitis.

Figure: A patient with diphtheria. Severe cervical lymphadenopathy and edema cause the "bull neck" appearance characteristic of this disease.

Figure: A child with parotitis (arrows) due to the mumps virus.

Figure: A photomicrograph of *Campylobacter* demonstrating the S-shaped (*black arrow*) and gull wing-shaped (*blue arrow*), gram-negative rod (Gram stain).

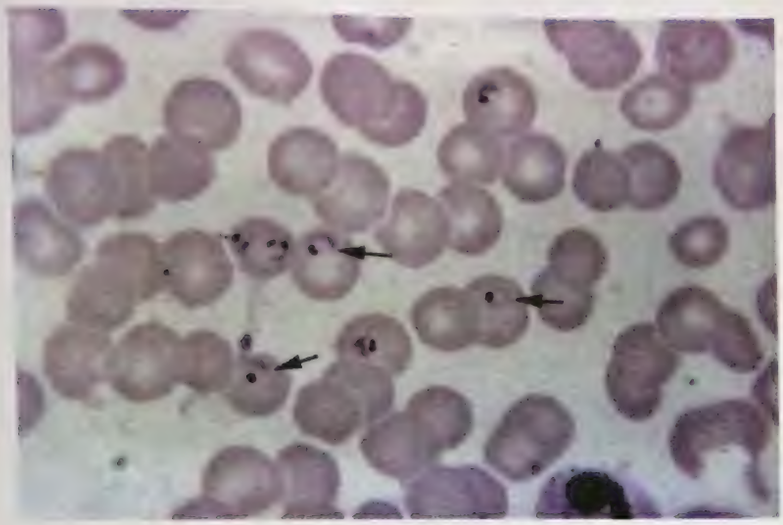


Figure: A photomicrograph of a Giemsa-stained thin blood film that reveals the ring-cell stage of the Plasmodium falciparum trophozoites (black arrow).

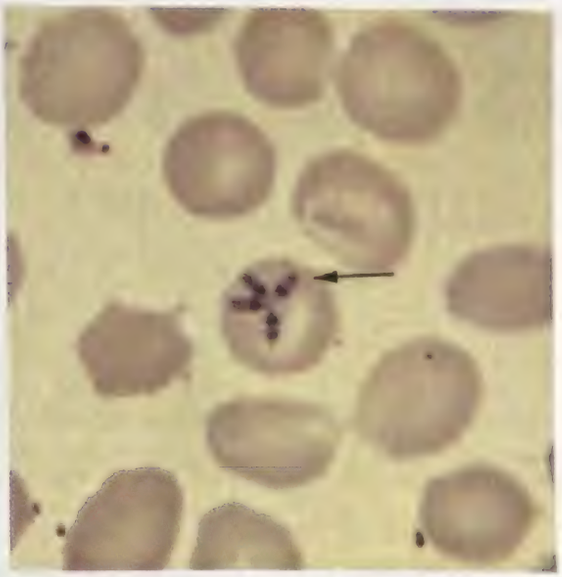


Figure: A photomicrograph of a Giemsa-stained thin blood smear containing the tetrad of trophozoites showing the Maltese cross (*black arrow*), characteristic of babesiosis.

Section - A

Revision at a Glance

- Basics of Bacteriology
- Basics of Virology
- Basics of Mycology
- Basics of Clinical Microbiology
- Culture and Sterilization
- Bacterial Genetics

CHAPTER

1

Basics of Bacteriology

CATALASE POSITIVE BACTERIA
• *Staphylococci*
• *N. meningitidis*
• *Atypical mycobacteria*
• *Pseudomonas*
• *Coliform*
• *H. influenza*
• *H. pylori*
• *Yersinia, Pasteurella*
• *Shigella except, • S. dysenteriae type I*
• *L. monocytogenes*
• *Nocardia*
• *Legionella*
• *Brucella except B.neotomae, B. ovis*

HEMOLYTIC ORGANISM	
• *Strep. pyogenes* - β	
• *S. aureus* - β	
• *Vibrio eltor*	
• *Clostridium perfringes*	
• *Bacillus subtilis*	
• *E. coli* (pathogenic strain)	
• *Mycoplasma*	
• *Corynebacterium mitis*	
• *Bacillus cereus*	
• *L. monocytogens* - β	
• Strep. viridans	α-**hemolysis**
• *Pneumococci*	
• Strep. sanguis	
• *Strep. mutans*	
• *Enterococcus*	

Transport media	Organism
Pike's media	S.pyogenes
Stuart's Media	Gonococci
Cary blair Media	V. cholera
Sach's buffered glycerol saline	*Shigella*

CAPSULATED BACTERIA
• ***P**neumococcus*
• ***B**acillus **a**nthrax*
• ***K**lebsiella*
• **H.** *influenza*
• ***Y**ersinia*
• ***B**ordetella*
• *N. **m**eningococci*
• ***Cl.** perfringes and butyricum*
• ***V.** parahemolyticus*
Mnemonic: **PAKIYB. M.C.V.**

TYPES OF MOTILITY		
• *Darting*	–	*V. cholera, Campylobacter*
• *Tumbling*	–	*Listeria at 20-25°C*
• *Stately*	–	*Clostridia*
• *Cork screw*	–	*T. pallidum*
Lashing	–	*Borrelia*
• *Gliding*	–	*Mycoplasma*
• *Swarming*	–	*Proteus mirabilis, P. vulgaris,*
	–	*Cl. tetani, Bacillus cereus*

MOTILE BACTERIA
Peritrichous flagella
• All clostridia except
Cl. perfringes and *Cl. tetani VI*
• *Bacillus* except *B. anthrax*
• *Listeria monocytogens*
• *E. coli*
• *Proteus*
• *Salmonella except, • S. gallinarum — pullorum*
Polar flagella
• *Vibrio*
• *Pseudomonas*
• *H. pylori*
• *Campylobacter*
• *Spirochetes*
• *Legionella*

ACID-FAST ORGANISM
• *Nocardia*
• *Legionella micdadei*
• *Smegma bacilli*
• *Bacterial spores*
• *Cryptococcus cyst*
• *Rhodococcus*
• *Isospora*
• *Mycobacterium*
• *Spermatic head*
Mnemonic: No Longer Separate Booking for RIM Sim Card

Shape of Bacteria		
• *Club shape*		Corynebacteria
• *Lanceolate* (flame shaped)		Pneumococci
• *Halfmoon* (Lens)		Meningococci
• *Kidney*		Gonococci
• *Comma*		V. cholera, *Campylobacter*

Pigment Producing Bacteria
• *Pseudomonas* — Green (*Ps aeroginosa*)
• *S. aureus* — Golden yellow • *Rhodococcus* — Red
• *Bacteroides melanogenicus* — Black
• *Nocardia* — Yellow to red
• Pepto and *Peptostreptococcus* • Photo and Scotochromogen — Yellow orange • Atypical mycobacteria • Hafnia, Serratia marcescens

PLEOMORPHIC ORGANISM
• *Mycoplasma*
• *Clostridium*
• *H. influenza*
• *V. cholera*
• *V. parahemolyticus*
• *Proteus*

DEAD END INFECTION
• *Leptospirosis*
• *Legionella*
• *Endemic typhus (Rickettsia typhi)*
• Tetanus (*Cl. tetani*)
• Human *rabies (Rhabdovirus)*
• Japanese encephalitis
• *T. solium*
• *Echinococcus granulosus and Trichinella spiralis*

AEROBIC BACTERIA
• *Bacillus anthrax I.*
• *Bordetella pertusis*
• *Brucella*
• *Klebsiella*
• *Listeria monocytogenes*
• *Mycobactena*
• *Nocardia*
• *Neiserria*
• *Pseudomonas*
• *Proteus*
• *Pasteurella group except Y. pseudo TB, Y. enterocolitica*
• *Vibrio cholera*
Mnemonic: B_3 KLMN$_2$ P_3V

ANAEROBES	
I. Cocci	
• Gram-positive	: *Peptococcus* *Peptostreptococcus*
• Gram-negative	: *Veilonella*
II. Bacilli	
• Endospores forming	: *Clostridia*
• Nonsporing:	
Gram-positive:	– *Eubacterium* – *Propionibacterium* – *Lactobacillus* – *Mobiluncus* – *Bifidobacterium* – *Actinomyces*
Gram-negative :	– *Bacteroides* – *Prevotella* – *Fusobacterium* – *Leptotrichia*
III. Spirochetes	– *Treponema* – *Borreli*

SPORE PRODUCING BACTERIA
• *B. anthrax and subtilis*
• *Sporosarcina*
• *Clostridia*
• *Coxiella burnetti*
Mnemonic: BSC Chemistry

BACTERIA IN PAIR
• *Neiserria*
• *Branhemella (Neisseria catarrhalis) and other Neiserria*
• *Pneumococcus*
• *Klebisella*

BIPOLAR STAINING = SAFETY PIN APPEARANCE
• *Hemophilus ducreyi*
• *Vibrio parahemolyticus*
• *Yersinia pestis*
• *Calymmatobacterium or Donovani granulomatis*
• *Pseudomonas mallei*
• *Pseudomonas pseudomallei*

INTRACELLULAR BACTERIA
• *Brucella and Bordetella*
• *Mycobacteria tuberculosis and leprae*
• *Legionella*
• *Rickettsia and Chlamydia*
• *Listeria monocytogenes*
• *Yersinia pestis*
• *Pneumococci*
• *Salmonella and Shigella*
• *Donovani granulomatis*
• *Neisseria meningococci and gonococci*

CASTANEDA'S STAINS
• *Rickettsiae*
• *Chlamydiae*

GIEMSA STAIN	
• *Rickettsiae*	• *Mycoplasma*
• *Chlamydiae*	• *Treponema pallidum*
• *Helicobacter pylori and Malarial parasite*	

UREASE POSITIVE
• *Proteus*
• *S. aureus*
• *Morganella*
• *Klebsella*
• *Nocardia*
• *Yersinia pseudotuberculosis, Y. enterocolitica*
• *Cryptococcus*
• *Diphtheroids*
• *Mycobacteria except MAC*
• *H. pylori*
• *Corynebacterium urealyticum*
Mnemonic: *PSM Ky NaYi Cory CD Meri Hai*

ORGANISM NOT GROW IN ARTIFICIAL MEDIA
• *M. leprae*
• *Rickettsiae*
• *Chlamydia*
• *Pathogenic treponemas*
• Virus
• Rhinosporidium

DESCENDING PARALYSIS
• Polio
• Tetanus
• Botulism
• Diphtheria

TOXINS INHIBITING PROTEIN SYNTHESIS
• Sh. dysenteriae I
• Diphtheria
• Pseudomonas
• Verotoxin = Shiga like toxin of *E.coli*

ACUTE PHASE REACTANT (APR)
• C - Reactive protein (CRP)
• Mannose binding protein
• Alpha-1-acid glycoprotein
• Serum amyloid P component
• ESR
• Platelets
• Ferritin
• I L-1
• TNF
• Coagulation protein
• Complement
• α1 antitrypsin
• Fibrinogen
• Haptoglobin
• Ceruloplasmin

CAUSES OF TRAVELLER'S DIARRHEA
• ***Enterotoxigenic Escherichia coli (MC)***
• *Enteroaggregative E. coli*
• *Shigella and enteroinvasive E. coli*
• *Salmonella*
• *Campylobacter jejuni*
• *Vibrio cholerae*
• *Rotavirus and Norwalk-like virus*
• *Entamoeba histolytica*
• *Giardia lamblia*
• *Cryptosporidium*
• *Cyclospora*

PULMONARY INFILTRATES IN IMMUNOCOMPROMIZED PATIENTS	
Infiltrate	***Causative organism***
Localized	*Bacteria, Legionella, Mycobacteria*
Nodular	*Fungi (e.g., Aspergillus or Mucor), Nocardia*
Diffuse	*Viruses (especially CMV), Chlamydia, Pneumocystis, Toxoplasma gondii, Mycobacteria*

INFECTIONS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION			
	Period after transplantation		
Infection site	**Early (< 1 month)**	**Middle (1-4 months)**	**Late (> 6 months)**
Disseminated	Aerobic gram-negative, gram-positive bacteria	Nocardia, Candida, Aspergillus, EBV	Streptococcus pneumoniae, Haemophilus influenzae, Neisseria meningitidis
Skin and mucous membranes	HSV	HHV-6	VZV
Lungs	Aerobic, bacteria, Candida, Aspergillus, HSV	CMV, Pneumocystis, Toxoplasma	Pneumocystis, Nocardia
Gastrointestinal tract	C. difficile	CMV	
Kidney		BK virus, adenovirus	
Brain		HHV-6, Toxoplasma	Toxoplasma, JC Virus
Bone marrow		CMV, HHV	

INFECTIONS AFTER KIDNEY TRANSPLANTATION			
	Period after transplantation		
Infection Site	**Early (< 1 month)**	**Middle (1-4 months)**	**Late (> 6 months)**
Urinary tract	Bacteria *(Escherichia coli, Kleibsella, Enterobacteriacea, Pseudomonas,* Enterococcus) associated with bacteremia; Candida	CMV (*fever, bone marrow suppression, hepatitis*); BK virus (*nephropathy, graft failure, vasculopathy*), JC virus.	Bacteria (late urinary tract infections usually not associated with bacteremia); BK virus (nephropathy, graft failure. generalized vasculopathy)
Lungs	Bacteria – *Legionella in* endemic settings)	CMV diffuse; pneumonitis, Legionella	Nocardia, invasive fungi
Central nervous system		*Listeria* (meningitis), Toxoplasma gondii	CMV *disease: Listeria (meningitis),* Cryptococcus (meningitis), *Nocardia*

SEXUALLY TRANSMITTED MICROORGANISMS		
Bacteria	**Viruses**	**Other**
Transmitted in adults predominantly by sexual intercourse		
Neisseria gonorrhoeae *Chlamydia trachomatis* *Treponema pallidum* *Calymmatobacterium granulomatis* *Ureaplasma urealyticum*	HIV (types1 and 2) Human T-cell lymphotropic virus type-I Herpes simplex virus type-2 Human papillomavirus Hepatitis B virus Molluscum contagiosum virus	*Trichomonas vaginalis* *Phthirus pubis*
Sexual transmission repeatedly described but not well defined or not predominant mode		
Mycoplasma hominis *Mycoplasma genitalium* Gardenerella vaginalis and other vaginal bacteria Group B *Streptococcus* *Mobiluncus* spp.	Cytomegalovirus Human T-cell lymphotropic virus type-2 Epstein-Barr virus Kaposi's sarcoma – associated herpesvirus Transfusion – transmitted virus	*Candida albicans* Sarcoptes scabiei

GASTROINTESTINAL PATHOGENS CAUSING ACUTE DIARRHEA				
Mechanism	**Location**	**Illness**	**Stool findings**	**Examples of pathogens involved**
Noninflammatory (enterotoxin)	Proximal small bowel	Watery diarrhea	No fecal leukocytes; mild or no increase in fecal lactoferrin	*Vibrio cholerae, Enterotoxigenic, Escherichia coli* (LT and ST), *Clostridium perfringens, Bacillus cereus, Staphylococcus aureus,* Rotavirus, Norwalk-like viruses, Enteric adenoviruses, *Giardia lamblia, Cryptosporidium spp., Microsporidia*
Inflammatory (invasion or cytotoxin)	Colon or distal small bowel	Dysentery or inflammatory diarrhea	Fecal polymorphonuclear leukocytes; substantial increase in *fecal* lactoferrin	*Shigella spp., Salmonella spp., Campylobacter jejuni, Enterohemorrhagic, E. coli, Enteroinvasive, E. coli, Yersinia enterocolitica, Vibrio parahaemolyticus, Clostridium difficile, Entamoeba histolytica*
Penetrating	Distal small bowel	Enteric fever	Fecal mononuclear leukocytes	*Salmonella typhi, Y. enterocolitica, Campylobacter fetus*

NORMAL BACTERIAL FLORA	
Skin	• *Staphylococcus epidermidis* • Staphylococcus aureus • Micrococcus species • Nonpathogenic *Neisseria species* • Alpha-hemolytic and nonhemolytic streptococci • Diphtheroids • Propoinibacterium species • *Peptostreptococcus species* • *Candida species, Acinetobacter species*
Nasopharynx	• Diphtheroids, Nonpathogenic *Neisseria* species, α- hemolytic streptococci; • *S. epidermidis,* Nonhemolytic streptococci, Anaerobes • Yeasts, *Hemophilus* species, pneumococci, *S. aureus*, gram-negative rods, *Neisseria meningitidis*
Gastrointestinal tract and rectum	• Various *Enterobacteriaceae* **except** *Salmonella, Shigella; Yersinia*; *Vibrio,* and *Campylobacter species* • Enterococci • Alpha-hemolytic and nonhemolytic streptococci • *Diphtheroids* • *S. aureus* in small numbers • Yeasts in small numbers • Anaerobes in large numbers (***MC*** *Bacteroides*)
Genitalia	• *Any amount of the following :* – *Corynebacterium species, Lactobacillus species,* α-hemolytic and nonhemolytic streptococci, Nonpathogenic *Neisseria species.* • *The following when mixed and not predominant :* – Enterococci, *Enterobacteriaceae* and other gram-negative rods, S. *epidermidis, Candida albicans* and other yeasts. – Anaerobes especially prevotella, *Clostridium* and *Peptostreptococcus species.*

TRANSPLACENTAL INFECTION		
• Toxoplasmosis	• Rubella	• CMV (***MC***)
• HSV	• Syphilis	• Varicella ZV
• Parvo B-19	• *Plasmodium*	• *T. cruzi*
• HIV	• Coxsackie virus	• Enteroviruses
• West Nile virus	• Measles	• Hepatitis B
• HCV	• TB	• Lymphocytic choriomeningitis virus

ONCOGENIC MICROBES AND PARASITES	
Organism	**Neoplasm**
Human papilloma virus (papovaviridae)	Cervical, vulvar, penile cancers, squamous cell carcinoma, oropharyngeal carcinoma
HSV type 2	Cervical carcinoma, B cell lymphoma
Hepatitis B virus (Hepadnaviridae)	Hepatocellular carcinoma
Hepatitis C virus (Flaviviridae)	Hepatocellular carcinoma, Lymphoplasmacytic lymphoma
HTLV - I (Retroviridae)	Adult T-cell leukemia/lymphoma
HTLV - I (Retroviridae)	T-cell variant of hairy cell leukemia
HTLV - III (Retroviridae)	AIDS related malignancies, NHL, Kaposi sarcoma, SCC (especially of urogenital tract), Diffuse large B-cell lymphoma, Burkitt's lymphoma
Epstein barr-Virus (Herpesviridae)	Mixed cellularity Hodgkin's, Nasopharyngeal carcinoma (anaplastic), African Burkitt's lymphoma, Post organ transplant lymphoma, Primary CNS diffuse large B-cell lymphoma, Extranodal NK/T cell lymphoma (nasal type)
H. pylori	Gastric malt lymphoma, Gastric cancer
Human Herpes virus 8	Primary effusion lymphoma, Multicentric Castleman's disease
Schistosoma hematobium	Bladder cancer (squamous cell)
Clonorchis	Cholangiocarcinoma
Opisthorchis	Cholangiocarcinoma

CHAPTER

2

Basics of Virology

DEFINITIONS

Virion	• Extracellular infectious virus particle
Capsid	• Protein coat that protects nucleic acid.
Envelope	• Lipoprotein coat which surrounds some virus particles. Lipid is of host cell origin while protein in the form of peplomers is virus coded.
Viroids	• Subviral infectious agent which is protein free and consist of low molecular weight RNA ***(mostly double stranded, small RNA)***. It is resistant to heat and organic solvents but sensitive to nucleases.
Prion	• Proteinaceous infectious particles causing chronic neurological degenerative disease of human

Virus is obligate intracellular parasite, without cellular organization and contains only one type of nucleic acid either DNA or RNA but never both. So classified as:

	CAPSID	VIRION	NUCLEIC ACID	VIRUS FAMILY	MEMBERS
DNA VIRUS					
I.	Icosahedral	Naked	SS (-ve)	Parvoviridae	B-19 parvovirus
II.	Icosahedral	Naked	ds circular (±)	Papovaviridae	Papilloma virus, JC, BK virus, polyomavirus
III.	Icosahedral	Naked	ds (±)	Adenoviridae	Human adenovirus
IV.	Icosahedral	Enveloped	ds with ss (±) circular	Hepadenoviridae	HBV
V.	Icosahedral	Enveloped	ds (±)	Herpesviridae	VZ; HSV I, II; CMV; EBV
VI.	Complex	Complex coats	ds (±)	Poxviridae	Variola (small pox) Molluscum contagiosum
RNA VIRUS					
I.	Icosahedral or (cubical)	Naked	SS (+)	Picornaviridae	Polio, coxsackie, entero, rhino, HAV
II.	Icosahedral	Naked	SS	Astroviridae	
III.	Icosahedral	Naked	SS (+)	Calcivridae	HEV, Norwalk
IV.	Icosahedral	Naked	ds segmented (±)	Reoviridae	Rota, Reo, Orbivirus
V.	Icosahedral	Enveloped	SS (+)	Togaviridae	Rubella virus
VI.	Unknown or complex	Enveloped	SS (+)	Flaviviridae	HCV, HGV, yellow fever, Dengue virus
VII.	Unknown or complex	Enveloped	SS (-) segmented	Arenaviridae (sandy appearance)	Lassa fever virus
VIII.	Unkonwn or complex	Enveloped	SS (+)	Coronaviridae	Corona virus
IX.	Unknown or complex	Enveloped	SS diploid (+)	Retroviridae	HIV 1, 2; HTLV I, II; slow virus group
X.	Helical	Enveloped	SS (-) segmented	Orthomyxoviridae	Influenza A, B, C
XI.	Helical		SS (-) segmented	Bunyaviridae	Hantavirus, sandfly fever virus
XII.	Helical	Enveloped	SS	Bornaviridae	
XIII.	Helical	Enveloped	SS (-)	Rhabdoviridae	Rabies virus, Vesicular stomatitis virus
XIV.	Helical	Enveloped	SS (-)	Paramyxoviridae	Parainfluenza, RSV, Mumps, rubeola New castle virus
XV.	Helical	Enveloped	SS (-)	Filoviridae	Marburg virus Ebola virus

Mnemonic:

- Segmented Nucleic acid
  = **'PARBO virus'** = Picornaviruses, Arena, Reo, Bunya, Orthomyxovirus
- Enveloped virus are sensitive to ether, chloroform, bile salts while non-enveloped are resistant
- All RNA virus are enveloped except **'PARC'** = Picorna, Astro, Reo, Calciviridae

- **Viruses with both DNA and RNA:** – Retrovirus
  – Lentivirus
  – HBV
- **Complex capsid:** – Pox
  – Bacteriophage
- **Shapes:**
  - *Bullet shaped* : Rabies virus
  - *Brick shaped* :– Pox virus
  - *Rod shaped* : Tobacco mosaic virus
  - *Space vehicle* : Adenovirus
- Smallest size virus : Parvovirus
- Largest size virus : Filoviridae > Pox viridae
- Virus with smallest genome: Circoviridae
- Virus with largest genome: Pandoraviruses

HEMATAGGLUTINATION (HA)

- It is agglutination of erythrocytes by virus.
- It is unstable in myxovirus because *Neuraminidase (RDE- receptor destroying enzyme)* cause reversal of hemagglutination called as ***Elution***. RDE also produced by cholera vibrios and many verterbrate cells.
- In other viruses HA is stable.
- In arbovirus, it is reversible by variation in pH and temperature.
- HA measures total quantity of virus.
- HA of *human* RBC is seen in - **R**eo, **I**nfluenza, **P**ara-influenza, **E**ntero and some cox and ECHO, **M**umps.
  Mnemonic: **RIPE Mango**
- HA also seen in measles, toga, rhino, rabies, pox, adenovirus.

POCK ASSAY

Used for quantitative infectivity assay of viruses [also done by plaque assay] since each infectious virus particle can form one pock, for example, variola, vaccinia, HSV, Pox (Monkey, Cow, Camel).

PHAGE ASSAY

Used for titrating number of viable bacteriophage and for purification of phages.

PHAGE TYPING

Used for typing and identification of bacteria, for example, Intraspecies typing of S. typhi (by using Vi antigen) and S. aureus; species specific bacteriophage of B. anthracis, MukerJee's phage IV for classical V. cholerae.

VIRUS MULTIPLICATION

- Critical step in viral biosynthesis is transcription of mRNA from viral nucleic acid.
- **DNA virus** synthesize nucleic acid in host cell **nucleus** except pox which synthesis all their components in host cell cytoplasm.
- **RNA virus** synthesize nucleic acid in **cytoplasm** except orthomyxo, some paramyxo and retrovirus which synthesize partly in nucleus.
- **Viral protein** is synthesized only in cytoplasm.
- Herpes and adeno assembled in nucleus while picoma and pox are assembled in cytoplasm.
- **Envelop:** Lipoprotein envelop, proteins are synthesized in cytoplasm whereas lipid is derived from cell membrane.

ABNORMAL REPLICATIVE CYCLE

Von Magnus Phenomenon: High hemagglutinin but low infectivity due to defective assembly or incomplete virus Eg. *Influenza virus.*

Abortive Infection: Defect in the type of cell (non-permissive cell) not in the parental viruses, which lead to defective maturation or assembly.

Defective virus: Genetically defective virus which are incapable of producing infectious daughter virions without the helper activity of another virus, e.g. Rous sarcoma virus, Hepatitis D Virus, adeno-associated satellite virus (dependovirus), Measles virus from SSPE, etc.

VIRAL INTERACTION

- *Genetic Interaction* – occur in virus by:
  - *Mutation:* Occur during every viral infection. Most mutation are lethal.
  - *Recombination:* Occur when two different but related viruses (both active or both inactive or one active and one inactive) infect a cell simultaneously. It leads to cross reactivation/marker rescue; multiplicity reactivation and formation of pseudovirion.
- Non-Genetic Interaction
  - Phenotyping mixing: transcapsidation occurs
  - Genotyping mixing
  - *Complementation*
  - *Interference:* Infection of a cell by one virus inhibits simultaneous or subsequent infection by other virus. Most important mediator is Interferon, a soluble cellular product.
    - It is applied in controlling polio outbreaks by introducing live attenuated polio vaccine.
    - It can be produced by receptor destruction as in myxo and enterovirus or by autointerference.

INCLUSION BODIES

It is the most characteristic histological feature in virus infected cells. It is of following types:

a. Intracytoplasmic eosinophilic inclusions:
   - ***Negri bodies*** – Rabies
   - ***Guarnieri bodies*** – Variola (small pox), vaccinia
   - ***Bollinger bodies*** – Fowlpox
   - ***Henderson - peterson bodies*** – Molluscum contagiosum

b. Intranuclear acidophilic inclusion bodies:
   - ***Cowdry type A*** – Herpes, Chicken pox, CMV, Yellow fever
   - ***Torres bodies*** – Yellow fever
   - ***Cowdry type B*** – Polio virus

c. Both Nuclear and cytoplasmic:
   - ***Warthin Finkeldey*** – Measles

d. Intranuclear basophilic inclusion bodies:
   - ***Cowdry type B*** – Adenovirus

RESPIRATORY VIRUSES

Viruses	Most frequent illness
Rhinoviruses	Common cold
Coronaviruses	Common cold
Respiratory syncytial virus	Pneumonia and bronchiolitis in young children
Parainfluenza viruses	Croup and lower respiratory tract disease in young children
Adenoviruses	Common cold and pharyngitis in children
Influenza A, B viruses	Influenza
Enteroviruses	Acute undifferentiated febrile illnesses
Herpes simplex viruses	Gingivostomatitis in children; pharyngotonsillitis in adults

VIRUS CAUSING LATENT INFECTION

• Measles	• Hepatitis B virus	• Hepatitis C virus	• Rabies virus
• Human T-lymphotrophic virus	• Herpes virus	• Kuru	• Oncogenic virus
• Scrapie	• Humanimmuno-deficiency virus		

REACTION TO PHYSICAL AND CHEMICAL AGENTS

- Stable at low temperature. So for long-term storage, they are kept by frozen at –70°C, by lyophilization or freeze drying but poliovirus do not stand freeze drying.
- All virus are disrupted under alkaline pH. Enterovirus are very resistant to acid pH while rhinovirus are very susceptible.
- Most active antiviral disinfectants are oxidising agents such as H_2O_2, $KMnO_4$ and hypochlorides.
- Chlorination kills most viruses except hepatitis virus and polio virus.

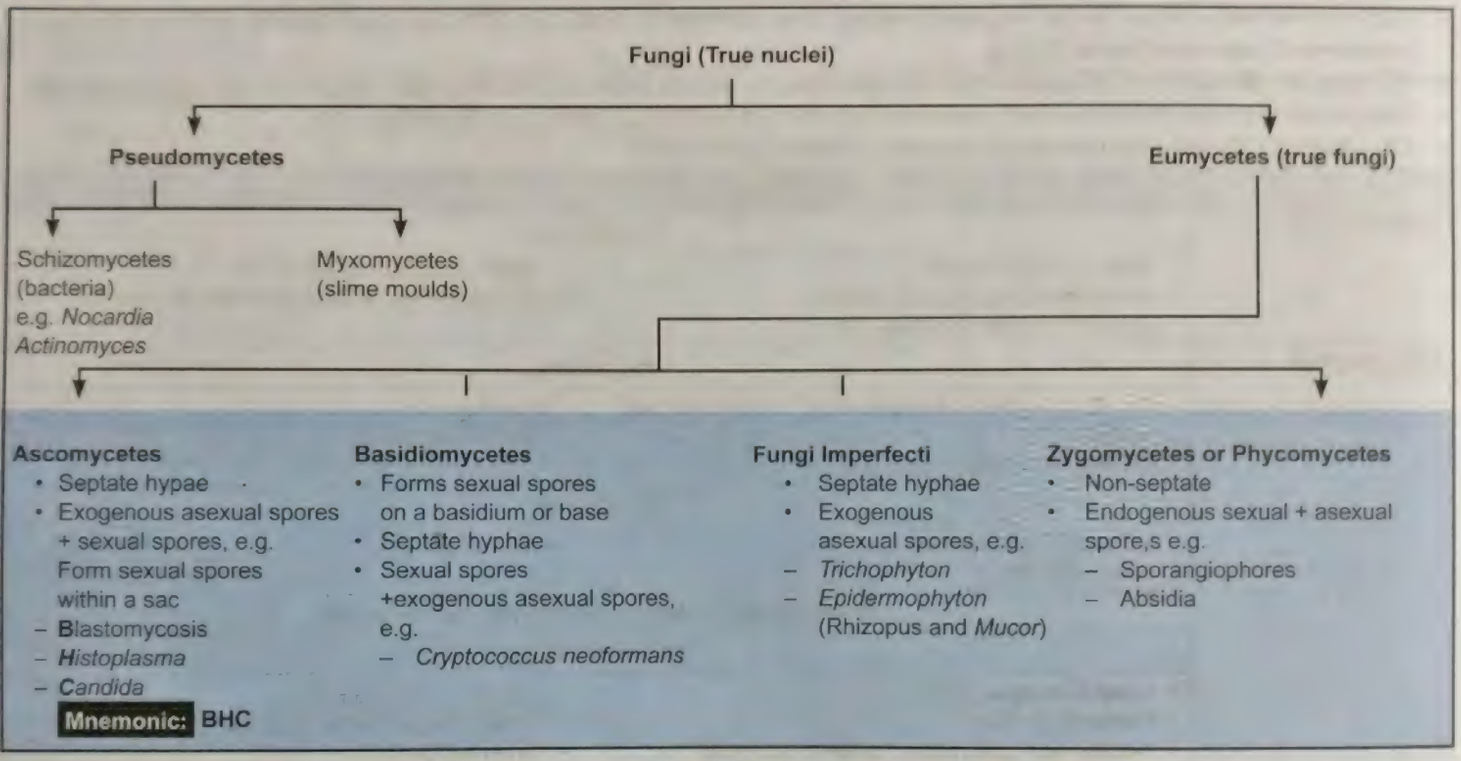
ONCOGENIC VIRUSES		
RNA VIRUSES	**Retroviruses**	
	• Avian leukosis viruses • Murine mammary tumour viruses • Human T-cell leukemia viruses	• Murine leukosis viruses • Leukosis-sarcoma viruses of various animals
DNA VIRUSES	**I. Papovavirus**	
	• Papillomaviruses of human beings, rabbits and other animals • BK and JV viruses	• Polyomavirus • Simian virus 40
	II. Poxvirus	
	• Molluscum contagiosum • Shope fibroma	• Yaba virus
	III. Adenovirus - *Not associated with human cancer*	
	IV. Herpes virus	
	• Marek's disease virus • Epstein-Barr virus • Herpes virus (pan, papio, ateles and saimiri)	• Lucke's frog tumour virus • Herpes simplex virus types 1 and 2 • Cytomegalovirus
	V. Hepatitis B and C viruses	

CHAPTER

3

Basics of Mycology

CLASSIFICATION OF FUNGI



- Endogenous Asexual spores are called **'Sporangiospores'** while exogenous asexual spores are called **'Conidia'**.

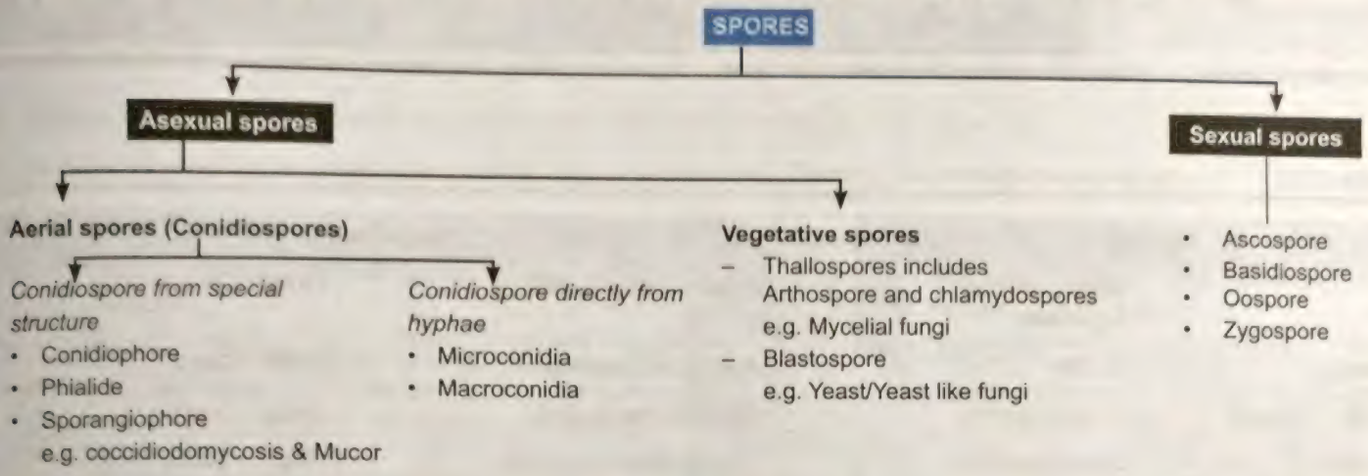
Fungal stains	
• Gomori methanamine silver (Best)	• Giemsa
• Gridley fungus	• Alcian blue
• Periodic acid schiff	• Meyer's Mucicarmine

Fungal medias
• Sabouraud's glucose agar
• Corn meal agar
• Czapek - Dox medium

MORPHOLOGICAL CLASSIFICATION			
Yeast	**Yeast like fungi**	**Moulds/filamentous or Mycelial fungi**	**Dimorphic fungi**
(No hyphae no mycelium) • Cryptococcus neoformans	**Only hyphae in the form of pseudomycelium** • Candida (forms blastopores) • Torulopsis (opportunistic)	**Hyphae + mycelium forms** • Dermatophytes (form arthospores) • Aspergillus • Zygomycetes • Fusarium • Cephalosoporium • Geotrichum • Scopulariopsis	• Candida albicans (not other candida) • Blastomyces dermatitidis • Paracoccidiodes brasiliensis • Coccidiodes immitis • Histoplasma capsulatum • Sporothrix schenckii • Penicillium marneffi

- Most fungi are soil saprophytes and human infection are mainly opportunistic.
- **Most** fungi causing *systemic infections* - Belong to Dimorphic fungi.
- **Most** fungi of medical importance belong to Fungi imperfecti group (Deuteromycetes or hyphomycetes).

- Aseptate fungi are called *Coenocytic fungi.*



FUNGAL DISEASE IN MAN		
Superficial mycoses • Pityriasis versicolor • Tinea nigra • White piedra • Black piedra	**Sub-cutaneous mycoses** • Mycotic mycetoma • Sporotrichosis • Chromoblastomycosis • Rhinosporidiosis • Subcutaneous phycomycosis • Phaeohyphomycosis	**Opportunistic** • Systemic candidiasis • Cryptococcosis • Aspergillosis • Mucormycosis (Zygomycosis) • Penicillosis
Cutaneous mycoses • Dermatophytes Microsporum, epidermophyton) • Candidiasis of skin, mucosa nails	**Endemic (primary, systemic)** • Coccidioidomycosis • Histoplasmosis • Blastomycosis • Paracoccidiomycosis	

CHAPTER

4

Basics of Clinical Microbiology

MENINGITIS

Etiology	
Age	**Most common organism**
< 1 month	*E. coli*
1 month - 18 years	N. meningitides
> 20 years	*S. pneumoniae*

Classification	
Streptococcus agalactiae	Gram+ Cocci
Streptococcus pneumoniae	Gram+ Cocci
Neisseria meningitidis	Gram - Cocci
Listeria monocytogenes	Gram + Bacilli
Hemophilus influenzae	Gram - Cocco Bacilli

COMMON SYMPTOMS

Headache

Fever

Chills

Nausea and Vomiting

Photo-phobia

Neisseria meningitidis

- MC cause in 2-20 years age group. Transmission is via respiratory droplets. Pili allow the attachment to the nasopharyngeal mucosa from where it enters the the blood stream causing meningococcemia. If it crosses the blood-brain barrier, it can infect the meninges, causing an acute inflammatory response that results in purulent meningitis.
- Presence of petechiae or purpurial rash provides an important clue.

Streptococcus pneumoniae

- MC cause in > 20 years of age. It is carried in the nasopharynx of many healthy individuals. Infection can be either *endogenous* (in a carrier who develops impaired resistance to the organism) or *exogenous* (by droplets from the airway of a carrier).
- *S. pneumoniae* infection can result in a bacteremia leading to infection of several sites in the human body, including the central nervous system. This meningitis has a high mortality rate.

Listeria monocytogenes

- It is common in neonates, pregnant women, elderly and in immunocompromised individuals for whom *Listeria is* one of the common causes of meningitis. *Listeria* infections are usually food borne, with the organism entering the body via the GI tract.
- Newborns can become infected during birth, resulting in meningitis with a significant mortality rate, intrauterine infections can cause the fetus to spontaneously abort or be stillborn.

Hemophilus influenzae

- It is a normal resident of the human upper respiratory tract. Transmission is by respiratory droplets.
- After attaching to the respiratory mucosa, the infection becomes systemic, with bacteria spreading via the blood to the central nervous system. It has been a leading cause of bacterial meningitis, especially in infants and very young children.

Streptococcus agalactiae

- It causes meningitis and septicemia in neonates. It is found normally in the vagino-cervical tract of female carriers, and the urethral mucous membranes of male carriers, as well as in the GI tract (especially the rectum). Transmission occurs during birth, and venereally among adults.
- Infection of an infant occurs as it traverses the birth canal. Its infection is a leading cause of neonatal meningitis, and has a high mortality rate.

URINARY TRACT INFECTION

Etiology
• ***Non-catheter associated (community acquired):*** *E.coli, Proteus, Klebsiella.*
• ***Obstruction or calculi associated:*** *E.coli, Proteus, Klebsiella, Serratia and Pseudomonas.*
• ***Catheter associated (nosocomial):*** *E.coli, Proteus, Pseudomonas, Serratia, Staphylococcus saprophyticus.*

COMMON SYMPTOMS

Escherichia coli

- *E.coli* is the most common cause of urinary tract infections (UTI), including cystitis and pyelonephritis. Women are particularly at risk for infection. Transmission is frequently from the patient's own flora.
- Uncomplicated cystitis (the most commonly encountered UTI) is caused by uropathogenic strains of *E. coli* characterized by *P. fimbriae* (an adherence factor). Complicated UTI (pyelonephritis) often occurs in settings of obstructed urinary flow, and may be caused by non-uropathogenic strains of *E.coli*.

Pseudomonas aeruginosa

- It is a major cause of hospital-acquired (nosocomial) infections such as UTI, particularly in patients who have been subjected to catheterization, instrumentation, surgery or renal transplantation, or to prior antibiotic therapy.
- Disease begins with attachment and colonization of host tissue. Pili on the bacteria mediate adherence, and glycocalyx capsule reduces the effectiveness of normal clearance mechanisms. Host tissue damage facilitates adherence and colonization.

Staphylococcus saprophyticus

- It is a frequent cause of cystitis in young sexually active women, probably due to its occurrence as part of normal vaginal flora. It is also an important agent of hospital-acquired infections associated with the use of catheters.
- It is a coagulase negative staphylococcal species. It is distinguished from other coagulase negative staphylococci by its *resistance to novobiocin*.

Dysuria
Lumbar Pain
Fever
Chills
Hematuria

Proteus and Klebsiella

- *Proteus spp.* by virtue of urease production, and *Klebsiella spp.* through the production of extracellular slime and polysaccharides, predispose to stone formation and are isolated more frequently from patients with calculi.

Other enterobacteria

- Other genera of *Enterobacteriaceae,* such as Enterobacter and Serratia, which can be found as normal inhabitants of the large intestine, are primarily opportunistic and often nosocomial pathogens. They all frequently colonize hospitalized patients, especially in association with antibiotic treatment, indwelling catheters, or invasive procedures, causing extraintestinal infections such as those of the urinary tract.
- These organisms produce exotoxins. Wide spread antibiotic resistance among these organisms necessitates sensitivity testing to determine the appropriate antibiotic treatment.

DIAGNOSIS

UTI is said when there is:	• Bacteriuria $\geq 10^5$ /ml in *asymptomatic*
	• Bacteriuria of $> 10^4$/ml in *symptomatic*
	• Bacteriuria of $\geq 10^2$/ml in *catheterized* sample
	• Bacteriuria of *any degree* in *suprapubic* aspirate

Pyuria in the absence of bacteriuria *(sterile pyuria)* may indicate infection with *C. trachomatis, U. urealyticum,* and *Mycobacterium tuberculosis* or with fungi.

FOOD POISONING

Etiology		
Onset : 1 - 6 hours	**Onset : 8 - 16 hours**	**Onset : > 16 hours**
• *Bacillus cereus (vomiting)* • *Staphylococcus aureus*	• *Clostridium perfringens* • Bacillus cereus (diarrhea)	• *Vibrio chloerea* • *ETEC* • *EHEC* • *Campylobacter jejuni* • *Clostridium botulinium* • *Escherichia coli* • *Salmonella species* • *Shigella species* • *Vibrio parahemolyticus*

COMMON SYMPTOMS

Staphylococcus aureus
MC cause of food poisoning in west.
- It is caused by ingestion of food containing the bacterial enterotoxin. Often contaminated by a food-handler, these foods tend to be protein-rich (e.g., egg, salad, cream, pastry).
- The toxin stimulates automatic nervous system by binding to neural receptors in the upper GI tract.

Diarrhea

Escherichia coli
- It is part of the normal flora of the colon, but can be pathogenic both inside and outside of the GI tract. Transmission is by contaminated food (such as beef and unpasteurized milk) and water serving as the vehicles.
- Several types of intestinal infections with *E.coli* have been identified.
  - *Enteropathogenic E. coli*
    - Diarrhea in infants and children
  - *Enterotoxigenic E. coli*
    - Traveller's diarrhea
  - *Enteroinvasive E. coli*
    - Diarrhea to dysentery similar to Shigellosis
  - *Enterohemorrhagic E. coli*
    - Hemorrhagic colitis
  - *Entero aggregative E. coli*
    - Persistent diarrhea

Nausea and Vomiting

Campylobacter jejuni
- Second MC cause of food poisoning in west. It also causes *traveller's diarrhea* and *pseudo-appendicits.*
- Transmitted to humans through exposure to contaminated meat (especially poultry).
- It typically causes an acute enteritis after incubation period of 1-7 days. Symptoms may be both systemic (fever, headache, myalgia) and intestinal (abdominal cramping and diarrhea, which may or may not be bloody)

Shigella species
- It causes shigellosis that occurs most commonly among young children.
- It invades and destroys the mucosa of the large intestine resulting in bacillary dysentery which is characterized by diarrhea with blood, mucus, and painful abdominal cramping.

GI Disturbances

Clostridium species
- *Cl. perfringens* food poisoning is caused by meat, chicken, fish. Typical episode involves cooking that fails to inactivate spores, followed by holding the food that allows bacterial germination and several cycles of growth.
- It secretes a cytotoxin which acts on small intestine brush border altering its permeability.
- Symptoms include diarrhea, abdominal cramp, nausea; fever is absent and vomiting is rare.
- *Cl. botulinum* causes food poisoning without diarrhea. It produces a neurotoxin that results in flaccid paralysis. Contact with the organism itself is not required.

Fever

Salmonella species
- Non-typhoidal *Salmonella*, particularly *S. typhimurium* and *S. enteritidis*, cause a localized gastroenteritis where the symptoms result from the causative bacteria proliferating in the intestine of affected individuals. Transmission is usually via food, especially chickens, eggs, and egg products.

Myalgia and Paralysis (in few cases)

CHAPTER

5

Culture and Sterilization

CULTURE MEDIAS

Type of medium	Name of medium	Laboratory use
Liquid media		
A. Basal (simple)	1. Peptone water	Routine culture, basal medium for sugar fermentation test
	2. Nutrient broth	Routine culture
B. Special (Complex)		
a. Enriched	1. Glucose broth	Blood culture
	2. Robertson's cooked meat medium	Culture of anaerobic bacteria
b. Enrichment	3. Tetrathionate broth	Enrichment culture for *Salmonellae*
	4. Selenite F broth	Enrichment culture for *Salmonella* & *Shigellae*
	5. Thioglycollate broth	Culture of anaerobic bacteria
	6. Alkaline peptone water	Enrichment culture for *Vibrio*
Solid media		
A. Simple	Nutrient agar (2-3% agar)	Routine culture
B. Special		
a. Enriched	1. Blood agar (also indicator media)	General culture, *Streptococcus;* **Most widely used medium**
	2. Chocolate agar	Culture of *H. influenzae, N. gonorrheae*
	3. Loeffler's serum	Culture of *C. diphtheriae*
	4. Dorsett's egg	Culture of *Mycobacteria*
b. Selective media (contains inhibitory substance)	1. MacConkey agar (also indicator and differential medium)	*Enterobacteria* (Lactose fermenters produce pink colonies)
	2. Deoxycholate citrate agar (DCA)	Selecitve medium for *Salmonella* and *Shigella*
	3. Lowenstein Jensen (LJ)	Culture of *Myco. tuberculosis*

Note: Agar is used in solid media due to its jellyfying property and it has no nutrient value.

I

The role of agar is to solidify the media.
It is used in 2%. concentration

I

Enriched Medium:
- Glucose broth (*Liquid*)
- Blood agar (*Solid*)
- Chocolate agar (*Solid*)
- Loeffler's serum (*Solid*)
- Dorsett's egg (*Solid*)

Selective Medium:
- MacConkey agar
- Deoxycholate citrate agar
- Lowenstein Jensen media

Indicator Media
- Wilson and Blair medium for *S.typhi.*
- Potassium tellurite in Mcleod's medium for *diphtheria bacilli.*

Culture Medias of Important Bacteria	
Media	**Bacteria**
MacConkey Agar	Enterobacteria
Selenite F broth	Shigella and salmonella
Thioglycollate broth	Culture of anaerobic bacteria
BCYE Agar	Legionella
New York city Agar	Neisseria gonorrhoea
Thayer-Martin Media	Neisseria gonorrhoea
LJ media	Mycobacterium

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Enriched Media:
- Substances such as blood, serum or egg are added to basal medium

Enrichment Media:
- Liquid media containing substance that can promote the growth of desired bacteria or inhibit the growth of unwanted one.

Selective Media:
- Solid media containing inhibiting substances so as to depress the growth of unwanted bacterias.

Some Important Culture Medias
- ***Robertson's Cooked Meat Medium:***
  - Used for anaerobes
  - Consist of nutrient broth and pieces of fat free minced cooked meat of ox heart.
  - Unsaturated fatty acids present in meat utilize oxygen for auto-oxydation. Certain reducing substances such as glutathione and cysteine also utilize oxygen.
  - With growth of saccharolytic bacteria (C. welchii) color of meat turns red while proteolytic bacteria (C. tetani) turns it black.
- ***MacConkey's Agar***
  - Selective and differential culture media for Gram-negative bacteria.
  - It contains bile salt, crystal violet dye, neutral red dye.
  - Crystal violet and bile salts inhibits the growth of Gram positive bacteria.

- *LJ Medium*
  - Specially used for Mycobacterium tuberculosis
  - Contains malachite green, glycerol, asparagine, potato starch.

STERILIZATION

Sterilization is the process by which article or medium is freed of all living microorganism either in the vegetative or spore state. Research of **Robert Koch** and his associates marks the beginning of science of disinfection and sterilization. Sterilizing agents are:

- Heat
- Radiation
- Filtration
- Sterilant gases, e.g. ethylene oxide
- Sterilant liquids.

DISINFECTION

Means destruction or removal of all pathogenic organism. Disinfecting agent (germicide) are:

- Substance interfere with membrane functions:
  - Surface active agents, e.g. quaternary ammonium compounds, tween - 80
  - Phenols, e.g. phenol, cresol
  - Organic solvents, e.g. chloroform, alcohols.
- Substance denaturing protein, e.g. organic acid, HCl, etc.
- Agents that destroy or modify functional group of proteins :
  - Heavy metals
  - Oxidizing agents, e.g. H_2O_2, chlorine, Iodine
  - Dyes, e.g. acriflovin, acridine
  - Alkylating agents, e.g. formaldehyde.

Heat

- Most reliable method of sterilization.
- It should be the method of choice unless contraindicated. It is of 2 types:

	Dry Heat	Moist Heat
Mechanism	Protein denaturation, oxidative damage and toxic effects of elevated levels of electrolytes	Denaturation and coagulation of proteins
Types	1. **Flaming** for sterilizing inoculating loop or wire tip of forceps and searing spatulas 2. **Burning or incineration** for contaminated cloth, animal carcasses and pathological materials, PVC and polythene (Polystyrene should autoclave) 3. **Hot air oven**: *Most widely used* method of sterilization by dry heat: • Holding period of 160°C for one hour is used to sterilize glassware, scalpels, forceps, scissors, all glass syringes, swabs, liquid paraffins, dusting powder, fats and grease. • For cutting instruments temperature of 150°C for 2 hours is required • **Drawback:** It has no penetrating power so not used for bulky articles such as mattresses	1. **Temperature below 100°C (for pasteurization of milk):** Holder method (63°C for 30 min) or **flash process** (72°C for 15 sec) destroy all nonsporing pathogens. Coxiella burnetii is relatively heat resistant and may survive holder method. Also used for vaccines of non-sporing bacteria, serum or body fluids containing coagulable proteins. **Inspissation:** Done between 75°C and 80°C. Purpose is to make the protein stiff without coagulation. Used for media containing serum or egg. 2. **Temperature at 100°C (Boiling):** Rolling boil (boiling for 5-10 minutes) will kill bacteria, but not spores or viruses 3. **Steam at atmospheric pressure 100°C:** Koch or Arnold steamer is usually used container and culture media are simultaneously sterilized. Single exposure of 20 min usually ensures sterilization; but for media containing sugars or gelatin an exposure of 100°C for 20 minutes on three successive days is used known as Tyndallization 4. **Steam under pressure = autoclave or steam sterilizer** (>100°C) - Most effective sterilizing agent for dressing, instruments, laboratory wares, media and pharmaceutical products; aqueous solutions. Sterilization control is by ***bacillus stearothermophilus***

I

Sterilization:
All organism are removed.

Disinfection:
- Pathogenic organism are removed

Moist heat sterilization:
- Pasteurization
- Boiling
- Tyndallization
- Autoclaving

I

- Sterilization control of dry heat is done by spore of non-toxigenic strain of Cl. tetani

I

Hot air oven

Temperature	*Duration*
160°C	120 min
170°C	60 min
180°C	30 min

I

- In autoclaving sterilization control is by bacillus stearothermophilus.
- Autoclaving is usually done at temperature of 121°C and chamber pressure of 15 psi for 15-20 minutes.

Note: Autoclaving is usually done at temperature of 121°C keeping chamber pressure of 15lb per square inch for 15-20 min. It can also be done at 126°C (20 lbs psi) for 10 min on 133°C (30 lbs psi) for 3 min.

Filtration

Used to remove bacteria; virus isolation; testing water samples for Vibrio cholera or Typhoid bacilli; and obtaining bacterial toxins. Membrane filters is routinely used in water purification and analysis, sterilization and sterility testing and for preparations of solutions for parenteral use. Most widely used pore diameter is 0.22 μm.

Radiation

Bacterial vaccines are best sterilized by filtration.

Non-ionization	Ionizing radiation
1. Infrared radiation - Form of *hot air sterilization* used for rapid mass sterilization of prepacked items such as syringes and catheters	• X-rays, gamma rays (Commonly used) and cosmic rays referred to *as cold Sterilization* • Used for plastics, syringes, swabs, catheters, animal feeds, cardboard, oils grease, fabrics, metal foils, musculo-skeletal grafts catgut suture
2. Ultraviolet radiation - For entryways, operation theaters and laboratories	• Most effective but very costly

I

Cold Sterilization:
- Sterilization by ionizing radiation

Alcohols

- **Most commonly** used are ethanol and isopropyl alcohol *(better)*.
- Both used as skin disinfectant in 70% concentration.
- Non-sporicidal but active against non-sporing bacteria and viruses.
- Isopropyl alcohol is used for disinfection of clinical thermometers.
- Methyl alcohol, effective against fungal spores and is used for treating cabinets and incubators.
- Most effective skin antiseptic is alcoholic solution of chlorhexidine and iodine.

Aldehydes

- *Formaldehyde:* – Sporicidal (Slow activity), bactericidal, virucidal. Used for sterilizing instruments and heat sensitive catheters and for fumigating wards, sick rooms and laboratories.
- *Glutaraldehyde:* – Specially effective for *tubercle bacilli*, viruses and fungi.
  – 2% solution called *Cidex* used for cystoscope and bronchoscopes, face mask, plastic endotracheal tubes, corrugated anaesthetic tubes

I

- Cidex is used for sterilization of fiberoptic scopes (cystoscope, bronchoscope)
- Formaldehyde for fumigation
- Ethylene oxide for heart lung machine

Dyes

- Acridine and aniline dyes used as skin and wound antiseptic.
- More active against Gram positive organism.

Halogens

- Iodine in aqueous and alcoholic solution is used widely as skin disinfectant.
- Active against tubercle bacteria, viruses, spores (moderately).
- **Iodophores**[Q] are compounds of iodine with non-ionic wetting or surface active agents. They are more active.
- Chlorine is used commonly as hypochlorites.

Ethylene Oxide

- Highly penetrating and highly active against all microorganism including viruses and spores[Q].
- Specially used for sterilizing **heart lung machine**[Q] and commonly used for sterilizing disposable plastic syringes.

I

Sporicidal Agents:
- Ethylene oxide
- Formaldehyde
- Glutaraldehyde
- Halogens

Formaldehyde Gas

- For fumigation of operation theaters and other rooms.
- Betapropiolactone is more efficient for fumigation than formaldehyde.

Surface Active Agents

- Most important antibacterial agents are cationic surface active agents.
- Larks action on spores, tubercle bacilli and most viruses.
- Most active at alkalines pH.
- Soaps prepared from saturated fatty acids are more effective against G–ve bacilli while those prepared from unsaturated fatty acids are more active against G+ve and neisseria group.

Plasma sterilization

- Plasma consist of ions, electrons or neutral particles
- For sterilization, radiofrequency energy is applied to create an electromagnetic field. Hydrogen peroxide vapors are then introduced in the electromagnetic field. This results in generation of plasma containing free radicals of hydrogen and oxygen.
- Plasma sterilization is highly efficient and can be used for heat susceptible instruments too.
- Clinically it is used for sterilization of arthroscopes, uretheroscopes, etc.

Resistance of a living form to antiseptic:
Prions (Most resistant) > coccidia > Spores > Mycobacteria > cysts > Virus (non enveloped)> Gram negative bacteria > Fungi > Gram positive bacteria > Enveloped virus (Most sensitive)

Germicidal properties of chemical disinfectants and antiseptics

Agent	Bacteria	Mycobacteria	Bacterial spores	Fungi	Viruses
Disinfectants					
Alcohol	+	+	-	+	+/-
Formaldehyde	+	+	+	+	+
Phenolics	+	+	-	-	+
Chlorine	+	+	+/-	+/-	+
Iodine (iodophors)	+	+/-	-	-	+/-
Hydrogen peroxide	+	+	+	+	+
Glutaraldehyde	+	+	+	+	+
Quaternary ammonium compounds	+	-	-	-	+/-
Antiseptic agents					
Alcohol	+	+	-	+	+
Iodophors	+	+	-	+	+
Chlorhexidine	+	+	-	+/-	+
Parachlorometaxylenol	+/-	+/-	-	+	-
Triclosan	+	+/-	-	-	-

Biological controls of different sterilization methods

Methods of sterilization	Biological control
Steam	Bacillus stearothermophillus
Hot-air oven	Bacillus subtilis subsp. niger
Autoclave	Bacillus stearothermophilus
Ethylene oxide	Bacillus globigii (a red-pigmented variant of Bacillus subtilis)
Ionizing radiations	Bacillus pumilus
Filtration	Serratia marcescens, B. diminuta
Formaldehyde	B. stearothermophilus

Based on the efficacy disinfectant can be:

- ***High level disinfectant***: Chemical that kills all microbial pathogens except large number of spores, e.g., glutaraldehyde and hydrogen peroxide. They are used for *sub-critical* items like endoscopes and bronchoscopes.

- ***Intermediate level disinfectant***: A chemical that kills all microbial pathogens including mycobacteria and non-enveloped viruses except spores, e.g., alcohol, phenolic compounds and iodophores. Used for some subcritical items like thermometer and BP cuffs for neonates.
- ***Low level disinfectant***: A chemical that kills only vegetative bacteria, fungi and lipid enveloped viruses. Used for non critical items like bed rails, BP cuffs, bed side tables.

Note:

Critical Items:	Equipments/instruments that enters sterile tissue, e.g. surgical instruments, intravenous catheters. They should undergo sterilization.
Subcritical Items:	Equipments which came in contact with mucous membranes/non-intact skin
Non-critical items:	These items came in contact with intact skin but not mucous membrane.

Remember:

Some common terms:

- *Biocide:* A chemical or physical agent, usually broad spectrum, which inactivates microogranism
- *Bacteriostatic:* Property by which a biocide inhibits bacterial multiplication. Multiplication resumes, after removal of agent
- *Bactericidal:* Property by which a biocide kills bacteria (fungicidal, sporicidal, virucidal are analogous tems)

Multiple Choice Questions

Culture

1. **Which of the following statement is true:** [AI 07]
   a. Solid media are enrichment media
   b. Nutrient broth is basal media
   c. Agar adds nutrient to media
   d. Chocolate agar is selective media

Sterilization and Disinfection

2. **Which of the following is most resistant to antiseptics?**
   a. Spore b. Prion [AI 08]
   c. Cyst d. Fungus

3. **A chest physician performs bronchoscopy in the procedure room of the outpatient department. To make the instrument safe for use in the next patient waiting outside, the most appropriate method to disinfect the endoscope is by:** [AI 03]
   a. 70% alcohol for 5 min
   b. 2% glutaraldehyde for 20 min
   c. 2% formaldehyde for 10 min
   d. 1% sodium hypochlorite for 15 min

4. **Heat labile instruments for use in surgical procedure can be best sterilized by:** [AI 03]
   a. Absolute alcohol
   b. Ultraviolet rays
   c. Chlorine releasing compounds
   d. Ethylene oxide gas

5. **All are true regarding disinfectants except:**
   a. Glutaraldehyde is sporicidal [AIIMS May 2011]
   b. Hypochlorites are virucidal
   c. Ethylene oxide is intermediate disinfectant
   d. Phenol usually requires organic matter to act

6. **The operating temperature in an ethylene oxide sterilization during warm cycle is:** [AIIMS 04]
   a. 20-35°C b. 49-63°C
   c. 68-88°C d. 92-110°C

7. **The sterilization method for catgut suture:** [PGI 2011]
   a. Steam b. Radiation
   c. Boiling d. Burning

8. **Choose the correct ones for the decreasing order of resistance to sterilization:** [PGI Dec. 07]
   a. Prions, bacterial spores, bacteria
   b. Bacterial spores, bacteria, Prions
   c. Bacteria, Prions, bacterial spores
   d. Prions, Bacteria, bacterial spores
   e. Bacterial spores, prions, bacteria

9. **Decreasing order of resistance to sterilization:**
   a. Spores, prions, non-lipid of small virus [PGI 07]
   b. Prions, spores, enveloped viruses
   c. Spores, mycobacteria, lipid or medium size virus

10. **Sterilising agents include:** [PGI 02]
    a. Dry heat b. Ethylene oxide
    c. Ether d. Alcohol
    e. Chlorohexidine

11. **Which of the following can be reliably used for hand washing:** [PGI 00]
    a. Chlorhexidine b. Isopropyl alcohol
    c. Lysol d. Cresol
    e. Glutaraldehyde

12. **Sporocidal agents are:** [PGI June 09]
    a. Glutarldehyde b. Ethylene oxide
    c. Formaldehyde d. Ether

13. **Disinfection of sputum is done by:** [PGI Dec 2008]
    a. Boiling b. Autoclaving
    c. Sunlight d. Burning
    e. Airing

14. **Radiation can be used to sterilize all except:**
    a. Bone graft b. Suture [AIIMS 2010]
    c. Bronchoscope d. Artificial tissue graft

15. **Which of the following is used as control. during plasma gas sterilisation:** [AIIMS 2010]
    a. B. Subtilis
    b. Stearothermophilus
    c. Cl. tetani
    d. Cl. Perfringens

16. **Sputum disinfection is done by all except:** [AIIMS Nov. 2010, May 2012]
    a. Autoclaving b. Cresol
    c. Boiling d. Chlorhexidine

17. **Which of the following is most resistant to the action of antiseptics and disinfectants:** [AI 2012]
    a. Spores b. Coccidia
    c. Prions d. Mycobacteria

18. **The indicator used in autoclave is:** [AIIMS May. 2012]
    a. Clostridium tetani
    b. Bacillus stereothermophilus
    c. Bacillus pumilis
    d. Bacillus subtilisavarniger

19. **Which is used in digestion and decontamination of sputum in smear preparation:** [PGI May 2013]
    a. NaOH b. KOH
    c. NaCl d. KCl
    e. N-acetyl-L-cysteine

20. **Which of the following are used for sterilization of surgical instrument:** [PGI May 2013]
    a. Ethylene oxide b. Gamma radiation
    c. Autoclaving d. Glutaraldehyde
    e. Hot air oven

Explanations and References with Illustrative Answers

1. **Ans. (b) Nutrient broth is basal media** *Ref. Ananthanarayan 8/e, p 40, 9/e, p 40*

MEDIA			
Simple media (basal media)	**Complex media**	**Synthetic or defined media**	**Special media**
• Nutrient broth • Nutrient agar	• Added ingredient	• Prepared from chemicals with defined composition e.g., simple peptone water medium	• *Enriched media* • Enrichment media • Selective media • Indicator media • Sugar media • Transport media

- **Enriched media :** Substance such as blood serum or egg are added to basal medium to promote growth, e.g. blood agar, chocolate agar, brain-heart infusion broth egg media.
- **Enrichment media :** In mixed culture usually the nonpathogenic or commensal bacteria tends to overgrow than pathogenic ones. In such circumstances, substances which has stimulating effect on pathogenic one or inhibitory effect on unwanted one are added to promote the growth of desired bacteria, if such substances are added to liquid medium. These media are called enrichment media, e.g. tetrathionate borth, selenite F broth, Alkaline peptone water.
- **Selective media:** As in enrichment media, if the inhibitory substance is added to solid medium, so as to supress the growth of unwanted one; the media is called *selective media* e.g., desoxycholate citrate medium.
- **Indicator media :** Changes colour on growth of bacteria e.g., Wilson blair media; *Mc*loed medium.
- **Differential media :** To differentiate different bacteria on the basis of characteristics, e.g. MacConkey's medium.
- **Sugar media :** – Here sugar means any fermentable substance.
  – Usual sugar media consist of 1% of the sugar in peptone water along with an appropriate indicator.
- **Transport media:** For delicate organism, e.g. Stuart media.

> **Remember Agar:** Contains long chain polysacchaaride (mainly), organic salts and small quantities of protein like substances. It has no nutritive value. Its main role is to solidify the culture media. In solid media it is used in 2% concentration.

2. **Ans. (b) Prion** *See below*

Resistance of organism to antiseptics in decreasing order is as follows :

Prions > Coccidia > Spores > Mycobacteria > Cysts > Small non-enveloped virus > Trophozoites > Gram-negative bacteria (non-sporulating) > Fungi > Large non-enveloped virus > Gram-positive bacteria > Lipid enveloped/medium size virus (HIV, HBV)

3. **Ans. (b) 2% glutaraldehyde for 20 min** *Ref. Ananth. 8/e, p 36, 9/e, p 34; Chakorvarty 2/e, p 46*

- 2% Glutaraldehyde also known as cidex (Aldehyde disinfectant), is specially effective against tubercle bacilli, fungi and viruses spores. It is most commonly used for cystoscope, endoscope, bronchoscopes, etc. which can't be disinfected by heat.
- Also used to disintect corrugated rubber anesthetic tubes and face masks, plastic endotracheal tubes, metal instruments and polythene tubing.

OTHER CHOICE		
• 70% alcohol is used as skin antiseptic • It acts by denaturing bacterial proteins • Methylated ethyl alcohol is MC alcohol preparation used for skin disinfection and hand washing	• 2-3 percent formaldehyde (20-30 ml of 40% formalin in one litre of water) is used for spraying rooms, walls, furniture and disinfecting blankets, beds, books • It is most effective at high temperature and relative humidity of 80-90%	• Sodium hypochlorite is recommended for sterilizing infant's feeding bottles • It acts in the same way as bleaching powder and is more strong.

Recommended Concentrations of Disinfectants Commonly used in the Hospitals	
Disinfectant	**Recommended concentration**
Ethanol	70% (700 gm/litre)
Methylated spirit	70% (700 gm/litre)
Glutaraldehyde	2% activated (available commercially as cidex)
Bleaching powder (calcium hypochlorite)	14 g/litre of water
Sodium hypochlorite	1% solution, 0.1% solution
Hydrogen peroxide	3% solution
Lysol	2.5% solution
Savlon	2.0%, 5.0%
Dettol	4.0%
Betadine	2.0

4. **Ans. (d) Ethylene oxide gas** *Ref. Park 22/e, p 121; Ananthanarayan 8/e, p 37, 9/e, p 35*

"Heat sensitive articles may be sterilized at 55-60°C by ethylene oxide which kills bacteria, spores, viruses."

- Ethylene oxide is highly penetrating and highly explosive.
- CO_2 or N_2 decrease its explosive tendency and water vapour increase its efficacy.
- It acts as alkylating agent.
- It is specially used for sterlizing heart-lung machines, sutures, dental equipment, books, fabrics, plastic equipment, cardiac catheters, clothing, glass, plastics, metal and paper surfaces.
- *There are two types of cycles during ethylene oxide sterilization:*
  - Cold cycle operates at 37±5°C
  - Warm cycle operates at 54±5°C

  Both cycles use Ethyle in oxide concentration of 700 mg/ltr. with relative humidity 40-50%
- It is unsuitable for fumigating room since it is explosive.

OTHER CHOICES		
Absolute alcohol	**UV rays**	**Chlorine releasing compounds**
• No action against spores • Recommended as rapidly drying disinfectant for skin and surface disinfection of clinical thermometers	• Used for disinfecting enclosed are as such as entry ways, hospital wards, operation theatres and laboratories	• **MC** used chlorine is hypochlorites • Used as antiseptics for dressing wound and for disinfection of water supplies, swimming pools, food/ dairy industries • They are bactericidal, virucidal

5. **Ans. is d i.e. Phenol usually requires organic matter to act** *Ref. Ananthanarayan 8/e p36; 9/e p35*

"Phenols are not readily inactivated by the presence of organic matter and are thus good general disinfectants".

—Ananthanarayan 9/e p 35

Phenols

- Obtained by distillation of coal tar between temperature of 170 and 270°C.
- Lethal effect is due to their capacity to cause cell membrane damage, precipitation of proteins and activation of membrane bound oxidases and dehydrogenases.
- As they are not inactivated by organic matter, phenol can be used as general purpose disinfactant.
- The related chlorphenols and chloroxyphenols are less irritant, active and more readily inactivated by organic matter.
- Lysol and cresol are other phenolic disinfactants.

6. **Ans. (b) 49 - 63°C** *Ref. Park 22/e, p 121*

Already explained

7. **Ans. (b) Radiation** *Ref. Ananthanrayan 8/e, p35, 9/e, p 33; Chakraborty 2/e, p 45-46*

Sterlisation of Important Materials	
Materials	**Methods of sterilisation and disinfection**
1. Glasswares–syringes, Petri dishes, test tubes, flasks, surgical instrument; oily fluids and powders	Hot air oven
2. Serum, body fluids, bacterial vaccines	Waterbath, vaccine bath
3. Milk	Pasteurisation, 63°C × 30 min. or 72°C × 20 sec
4. Cystoscope and endoscope	Glutaraldehyde (Cidex-2%) or ethylene oxide
5. Most of the culture media	Autoclaving
6. Culture media containing egg, serum or sugar	Tyndallisation
7. Rubber, plastic and polythene tubes, disposable syringes	Glutaradehyde, ethylene oxide gas
8. Dressings, aprons, gloves, catheters surgical instruments except sharp instruments.	Autoclaving
9. Sharp instruments	5% cresol
10. Suture materials except catgut	Autoclaving
11. Catgut	Ionising radiation
12. Rubber or plastic disposable goods, disposable syringes, bone and tissue graft, adhesive dressings	Ionising radiation
13. Faeces and urine, vomitus sputum	Bleaching powder, cresols, formalin, burning, autoclaving, boiling
14. Sterilisation of operation theatre	Formaldehyde gas
15. Wards and laboratory or operation theatre floor space	Formaldehyde gas and cresols (Lysol)
16. Skin	Tincture iodine, spirit (70% ethanol), Savlon (phenol derivative).

8. **Ans. (a) Prions, bacterial spores, bacteria**
Already explained

9. **Ans. (b and c) Prions, spores, enveloped viruses and Spores, mycobacteria, lipid or medium size virus**
Already explained

10. **Ans. (a and b) Dry heat and Ethylene oxide** *Ref. Chakraoborty 2/e, p 35, 41*
Sterilization is the process by which an article, surface or medium is *freed of all living microorganism* either in vegetative or spore state.

Method of Steriliation		
Physical sterilants	**Gas Vapour sterilants**	**Chemical sterilants**
• Steam under pressure	• Ethylene oxide	• Glutaraldehyde
• Dry heat	• Formaldehyde vapour	• Peracetic acid
• Filtration	• Chlorine dioxide gas	
• UV radiation	• Hydrogen peroxide vapour	
• Ionizing radiation	• Plasma gas (highly ionized H_2O_2)	

Disinfection means"destruction or removal of ***all pathogenic organism*** which are capable of producing infection.

Disinfecting Agents	
• Heat (moist heat)	• Alcohols (ethyl and isopropyl alcohol)
• Dyes	• Halogens (Iodine, chlorine)
• Phenol derivatives (hexachlorophene, lysol, cresol)	• Biguanides (chlohexidine)
• Oxidising agents ($KMnO_4$, H_2O_2) peracetic acid.	• Quaternary ammonium compounds (cetrimide)
• Soaps	• Acids
• Aldehydes (formaldehyde, glutaraldehyde)	• Metallic salts
• U.V. radiation	

Remember: Ether is used as anaesthetic agent not as disinfectant or sterilizing agents.

11. Ans. (a), (b) and (d) Chlorhexidine, Isopropylalcohol and Cresol *Ref. KDT 6/e, p 858-861; Chakarvarty 2/e, p 46*

SKIN DISINFECTANTS	
i. Chlorhexidine (Savlon)	vi. Cresol
ii. Alcohols (as spirit) - Isopropyl alcohol (preferred), ethyl alcohol	v. Chloroxylenol (Dettol)
iii. Iodine	vi. Hexachlorophene
	vii. Tincture iodine

12. Ans. (a), (b) and (c) Glutarldehyde, Ethylene oxide, Formaldehyde *Ref. Ananthanarayan 7/e, p 32; Park 19/e p 110*

Sporicidal Agents:

E – Ethylene oxide
F – Formaldehyde
G – Glutaraldehyde
H – Halogens (moderate action on spores)

- Benzalkonium chloride is quaternary ammonium cationic, antiseptic which are not sporicidal. ...*KDT 6/e, p 860*
- Phenol and hexachlorophene are poor sporicidal

13. Ans. (a, b and d) Boiling, Autoclaving, Burning *Ref. Park 22/e, p 122*

Disinfection of sputum

- Burning (best)
- If amount is considerable as in TB, then disinfection is done by either boiling or autoclaving for 20 minutes at 20 lbs pressure or by 5% cresol.

14. Ans. (c) Bronchoscope *Ref. Ananthanarayan 7/e, p 35, 36; 9/e, p 34*

- Rigid bronchoscopes are sterilized by autoclaving where as flexible bronchoscope and other flexible endoscopes are
- sterilized by cidex (2% solution of glutarldehyde)
- Bone graft and other salt tissue allograft (achilles tendon, fascialata) are sterilized by gamma irradiation.

Technique	Applications
Ionizing radiation	Rubber or plastic disposable items, bone grafts, tissue grafts, surgical catgut, culture media
Non ionizing radiation	• Infrared radiation for rapid mass sterilization of syringes • Ultraviolate radiation for disinfection of OT, laboratories, entry ways.
Glutaraldehyde	Flexible endoscopes, face mask, anaesthesia breathing circuits, reusable plastic endotracheal tubes

Note:

- Red ruber (reusable) endotracheal tubes are sterilized by autoclaving
- Plastic endotracheal tubes (reusable) are sterilized by cidex (2% glutaratdehyde)
- PVC endotracheal tubes (disposable) are sterilized by ionizing radiation
- Spores and Gram positive bacteria are resistant to UV rays, that's why disinfection (not sterilization) is used in context to UV rays.

15. Ans. (b) B. Stearothermophilus *Ref. Chakarvorty 2/e 41; Principles and practice of Disinfection and sterilization by A.P. Fraise, PA Lambert 4/e, p 423-428*

Plasma Gas sterilization

- This is a type of chemical sterilization using hydrogen peroxide
- Hydrogen peroxide is vaporized which is then converted into plasma. For sterilizatiion only cold plasma is used.
- When H_2O_2 vapours are energized into plasma, hydroxyl and hydroperoxyl free radicals are generated along with production or UV rays.
- Microbicidal activity is via three mechanism:
  - Direct destruction by UV radiation
  - Free radical induced damage
  - Direct erosion of cells
- The Sterlization cycle of plasma is divided into five steps, viz:
  - Evacuation phase
  - Injection phase (until a 6 mg/L H_2O_2 level is obtained)
  - Diffusion phase

- Plasma phase
- Re-establishment of atmospheric pressure.

- This plasma kills all spores, bacteria, fungi, virus. However prions are not killed at all.
- Plasma sterilization is an alternative to ethylene oxide and is used for heat and water sensitive equipments.
- ***Sterrad sterilizers*** is the only plasma gas sterilizer currently approved by FDA.

Control: Bacillus stearothermophilus spores are used as biological indicator.

16. **Ans. (d) Chlorhexidine** *Ref. Park 22/e, p 120, 122*
Already explained.

17. **Ans. (c) Prions** *Ref. Ananthanarayan 8/e p 552*
Already explained

18. **Ans. (b) Bacillus stereothermophilus** *Ref. Ananthanarayan 8/e p 34; 9/e p 32*

Autoclave Quality Assurance
Chemical and biological indicators are used to ensure than an autoclave reaches the desired temperature for the correct amount of time:

(a) **Chemical Indicators:**
- These indicators change colours when exposed to temperature equivalent to the autoclave desired temperature
- Browne's tube, autoclave tapes are the chemical indicators commonly used.
- Bowie dick tapes are used to test the vaccum obtained in the equipment.

(b) **Biological Indicators:**
- For determining the efficacy of autoclaving and other moist heat sterilization, spores of *Bacillus stearothermophilus* are used as test organism.
- *B. Stearothermophilus* is a thermophilic organism with an optimum growth at 55 to 60°C. Spores of *B. stearothermophilus* require an exposure of 12 minutes at 121°C to be killed.
- Paper strips impregnated with 106 spores are dried at room temperature and placed in paper envelope, along with the material to be sterilized.
- After sterilization, the strips are inoculated at 55°C for five days, if growth is positive, than autoclave is faulty.

19. **Ans. (a, e)** NaOH, N-acetyl-L-cysteine *Ref. Ananthanarayan 9/e p 352*

Decontamination of sputum
- **Petroff's method**
  - Most commonly used method, here sputum is incubated with on equal volume of 4% NaOH solution at 37°C with frequent shakings till it become clear. It is then centrifuged and the sediment is neutralized with N/10 HCl
- **N. acetylcysteine combined with 2% NaOH:**
  - This method is better than Petroff's. Here N acetylcysteine is used for liquification of sputum. NaOH kills the contaminating bacteria. The sample is then neutralized with buffer and concentrated by centrifugation.

20. **Ans. (c, d, e)** Autoclaving, Glutaraldehyde, Hot air oven *Ref. Chakarvorty 2/e 45, 46*
Already explained see Answer No. 7

Chapter Review

1. **Glassware is sterilized by?** [DNB 2013]
   a. Boiling
   b. Hot air oven
   c. Heating
   d. None
   [Ref. Chakravorty 2/e, p 39]

2. **Autoclaving is done in :** [Kolkata 02]
   a. Dry air at 121°C and 15 lb pressure
   b. Steam at 100°C for 30 minutes
   c. Steam at 121°C for 30 minutes
   d. Dry air at 160°C for 30 minutes
   [Ref. Chakraborty 2/e, p 38]

3. **The disposable plastic syringes are commonly sterilized by:** [DNB 2013]
   a. Formaldehyde
   b. Ethylene oxide
   c. Hexachloride
   d. UV radiation
   [Ref. Ananthanarayan, 9/e, p 35]

4. **Glassware and syringes are sterilized by:** [Kolkata 03]
   a. Autoclave
   b. Hot air oven
   c. Ethylene oxide
   d. Irradiation
   [Ref. Chakarborty 2/e, p 45]

5. **pH of Sabourauds dextrose agar is adjusted to :** [Kar 04]
   a. 4-6
   b. 1-2
   c. 6-8
   d. 8-10
   [Ref. Ananthanarayan, 8/e, p 601; 9/e p 592]

   pH of Sabouraud's agar is 5.4

6. **Asepsis means :** [Kolkata 04]
   a. Absence of pathogenic microbes
   b. Disinfection of the surface
   c. Prevention of infection
   d. Destroying all forms of microorganism
   [Ref. Jawetz 25/e, p 58]

7. **Mac Conkeys' Agar medium is :** [UP 06]
   a. Enriched medium
   b. Enrichment medium
   c. Differential medium
   d. Synthetic medium
   [Ref. Ananthanarayan 8/e. 7/e, p 37]

8. **Gamma radiations are used for sterilizing:** [UP 06]
   a. Syringes
   b. Cystoscopes
   c. Dressing aprons
   d. Metal instruments
   [See list of our book]

9. **Sterilization of fibre optic bronchoscope is done by?**
   a. Glutaraldehyde
   b. Chlorine [DNB 2012]
   c. Autoclave
   d. Phenol
   [Ref. Ananthanarayan, 9/e, p 34]

10. **Out of the following the true statement regarding sterlization is:** [AI 97]
    a. Dry heat is the best method of sterilization of liquid paraffin
    b. All glass syringes are best sterilized by boiling at 100°C
    c. Bacterial vaccines are best sterilized by ethylene oxide
    d. Pasteurization of milk by flash method is done by heating at 63°C for 30 minutes
    [Ref. Ananthanarayan, 8/e, p 31]

11. **Blood agar is an example of:** [DNB 2012]
    a. Enriched media
    b. Enrichment media
    c. Nutrient media
    d. Special media

12. **Conditions required for autoclave is:** [DNB 2012]
    a. 121°C temperature for 20 min
    b. 121°C temperature for 15 min
    c. 100°C temperature for 60 min
    d. 100°C temperature for 90 min
    [Ref. Chakarborty 2/e, p 39]

13. **Sterilization of culture media containing serum is by:**
    a. Autoclaving
    b. Micropore filter [PGI 98]
    c. Gamma radiation
    d. Centrifugation
    [Ref. Ananthanarayan, 8/e, p 32; 9/e p 37]

- Most of culture media are sterilized by Autoclaving at 121°C for 15 min at 15 lb pressure/inch^2.
- **Steam under pressure** *(Autoclaving or steam sterlizer).* Used for sterilizing dressings, instruments, laboratory ware, media, pharmaceutical products and aqueous solution.

Answers 1. b. Hot air oven 2. c. Steam at ... 3. b. Ethylene oxide 4. b. Hot air oven 5. a. 4-6
6. a. Absence of... 7. c. Differential ... 8. a. Syringes 9. a. Glutaraldehyde 10. a. Dry heat...
11. a. Enriched media 12. a. 121°C temp... 13. a. Autoclaving

NEET Pattern Questions

1. **Which is enrichment media:**
   a. Selenite F broth
   b. Chocolate media
   c. Meat extract media
   d. Egg media
   [Ref. Ananthanarayan, 9/e, p 42]

Selenite F is an enrichment media for Sh. dysentry

2. **In nutrient agar concentration of agar is:**
   a. 1
   b. 2%
   c. 3%
   d. 4%
   [Ref. Ananthanarayan, 9/e, p 40]

Nutrient agar is made by adding 2% agar to nutrient broth. Nutrient agar is the simplest and most common culture medium in routine

3. **Blood agar is an example of:**
   a. Enrichment media
   b. Indicator media
   c. Enriched media
   d. Selective media
   [Ref. Ananthanarayan, 9/e, p 40]

Cell Culture

Primary Cell culture	Diploid Cell strains	Cutaneous Cell Lines
• Normal freshly taken cells e.g. – Monkey kidney – Human amnion – Chick embryo cell culture	• Cells that retain the original diploid chromosome number and karyotype • Beside viral culture these cells lines are used for production of viral vaccine Example: Human fibroblast	• Derived from cancer cells, they are capable of continuous serial cultivation indefinitely e.g. HeLa HEp-2 KB Cell lines McCoy

Blood agar is a solid culture medium consisting of agar, peptones and blood

4. **Robert Koch assistant advised him to use agar instead of gelatine culture media for cultivation of bacteria as:**
   a. Agar has more nutrition
   b. Gelatin melts at 27°C
   c. Gelatin is not easily available
   d. Agar is cheaper
   [Ref. Ananthanarayan, 9/e, p 39]

Gelatin liquids at 24°C whereas agar melts at 98°C

5. **Endoscope tube is sterilized by:**
   a. Glutaraldehyde
   b. Formalin
   c. Autoclaving
   d. Boiling
   [Ref. Ananthanarayan, 9/e, p 34]

6. **Brown's tube is used for:**
   a. Steam sterilization
   b. Radiation
   c. Chemical sterilization
   d. Filtration
   [Ref. Ananthanarayan, 9/e, p 32]

7. **Which of the following is a primary cell culture:**
   a. Chick fibroblast
   b. Hela
   c. HEP-2
   d. HL-8
   [Ref. Ananthanarayan, 9/e, p 436]

8. **Prions are best killed by:**
   a. Autoclaving at 121°C
   b. 5% formaline
   c. Sodium hydroxide
   d. Sodium hypochloride
   [Ref. Ananthanarayan, 9/e, p 442; Harrison 18/e, p 3446]

Autoclaving at 134°C for 5 hours or treatment with NaOH in 2N concentration is recomended for sperilization of prions.

9. **Human fibroblast cell line is used for cultivation of:**
   a. Adenovirus
   b. Poliovirus
   c. HIV
   d. Measles
   [Ref. Ananthanarayan, 9/e, p 435]

Diploid cell strain, Human fibroblast is used for cultivation of coxsackie, CMV, enterovirus, echovirus, HSV 1-2, Poliovirus, VZV.

Answers 1. **a.** Selenite F broth 2. **b.** 2% 3. **c.** Enriched media 4. **b.** Gelatin melts at 27°C
5. **a.** Glutaraldehyde 6. **a.** Steam sterilization 7. **a.** Chick fibroblast 8. **c.** Sodium hydroxide
9. **b.** Poliovirus

10. Chocolate agar is:
a. Basal medium
b. Enrichment medium
c. Enriched medium
d. Simple medium

[Ref. Ananthanarayan, 9/e, p 40]

Enriched media: Brain-heart infusion broth/agar, blood agar, chocolate agar, egg media.

11. Simple based meida is:
a. Simple nutrient agar
b. Alkaline peptone water
c. Glucose broth
d. Blood agar *[Ref. Ananthanarayan, 9/e, p 40]*

Nutrient agar made by adding 2% agar to nutrient broth is simplest and most common medium in routine microbiology

12. Triple iron sugar medium contains all, except:
a. Lactose
b. Sucrose
c. Glucose
d. Maltose

Triple sugar iron agar (TSI) is a differential medium that contains lactose, sucrose and a small amount of glucose. It is used to differentiate enteric organism based on the ability to reduce sulfur and ferment carbohydrates.

13. Ideal urine specimen for anerobic culture should be:
a. Mid stream urine sample
b. First few drops at morning
c. Sample by foley's catheter
d. Sample by suprapubic aspiration

[Ref. Ananthanarayan, 9/e p 671]

14. Not an enrichment medium:
a. Selenite F broth b. Tetrathionate broth
c. Alkaline peptone water
d. Loeffler's serum

[Ref. Ananthanarayan, 9/e, p 42]

15. All selective media are correctly matched except:
a. V cholerae-TCBS medium
b. Pseudomonas-Cetrimide agar
c. M tuberculosis-LJ medium
d. Campylobacter-BCYE medium

BCYE is the slectine media for legionella.

16. Best specimen for anaerobic culture:
a. Exudates from wound
b. Pus aspirated in vial
c. Swab from wound
d. Mid-stream urine

Best specimen for anaerobic culture is an aspirate obtained by needle & syringe, this is to prevent exposure of air.

Anaerobic specimen collection:
- Disinfect skin surface with 70% alcohol, allow to dry
- Aspirate specimen directly in to the syringe
- If unable to aspirate, infect 0.9% saline and repeat aspiration
- Remove air from syringe
- Aseptically transfer material into an anaerobic transport vial for fluids

17. Aerobic blood culture should be incubated for how many days, before discarding:
a. 2 days
b. 3 days
c. 10 days
d. 14 days

Incubation time
48 hours : Aerobic
72 hours : Anaerobic
7 days : Final report negative culture

18. Differential media is:
a. Nutrient agar
b. Chocolate agar
c. MacConkey's agar
d. Tetrathionate broth

[Ref. Ananthanarayan, 9/e, p 42]

19. Simple Basal media is:
a. Simple nutrient agar
b. Alkaline peptone water
c. Glucose broth
d. Blood agar

[Ref. Ananthanarayan, 9/e, p 40]

20. Laparoscope is sterilized by:
a. 2% formaline b. 2% glutaraldehyde
c. Autoclaving d. Boiling

[Ref. Ananthanarayan, 9/e, p 34]

21. Gamma irradiation used for all of the following except:
a. Syringes
b. Catgut suture
c. Grafts
d. Endoscope *[Ref. Ananthanarayan, 9/e, p 33]*

22. Sterilization is defined as:
a. Disinfection of skin
b. Complete destruction of all microorganisms
c. Destruction of pathogenic organisms
d. Decrease bacterial count from objects

[Ref. Ananthanarayan, 9/e, p 28]

Answers **10.** b. Enrichment... **11.** a. Simple nutrient... **12.** d. Maltose **13.** a. Mid stream... **14.** d. Loeffler's serum
15. d.Campylobacter-BCYE **16.** b. Pus aspirated... **17.** b. 3 days **18.** c. MacConkey's...
19. a. Simple nutrient... **20.** b. 2% glutaraldehyde **21.** d. Endoscope **22.** b. Complete...

23. Cold sterilization is:
a. Sterilization by negative temperature
b. Sterilization by ionizing radiation
c. Sterilization by liquid CO_2
d. Sterilization by non-ionizing radiation
[Ref. Ananthanarayan, 9/e, p 33]

24. Seitz filter is a:
a. Candle filter
b. Asbestos filter
c. Membrane filter
d. Sintered glass filters
[Ref. Ananthanarayan, 9/e, p 33]

Types of filters.
(a) *Candle filter*: Mainly used for purification of water for industrial and drinking purpose. E.g. Doulton and Chamberlon
(b) *Asbestos filters*: Disposable single use. The carcinogenic potential of asbestos has discouraged their use. E.g. Seitz and sterimat.
(c) *Sintered glass filter*: They are brittle and expensive
(d) *Membrane filters*: Made of cellulose esters. They are routinely used in water purification and analysis and have replaced other types of filters.

25. Best virucidal disinfectant is:
a. Phenol
b. Hypochlorte
c. BPL
d. Formaldehyde

26. Lethal effect of dry heat is due to:
a. Denaturation of proteins
b. Oxidative damage
c. Toxicity due to metabolites
d. All of the above *[Ref. Ananthanarayan, 9/e, p 29]*

27. New York agar is used for:
a. Salmonella
b. Clostridia
c. Neisseria
d. Bacillus Anthracis *[Ref. Ananthanarayan, 9/e, p 40]*

New York city Agar, a peptone corn starch agar base buffered with phosphates supplemented with horse plasma, horse haemoglobin, dextrose and antibiotics. Peptone, haemoglobin provide nutrients for the growth of N. gonorrhoeae.

28. Example of selective medium is:
a. LJ medium
b. Blood agar
c. Selenite F broth
d. Chocolate agar *[Ref. Ananthanarayan, 9/e, p 42]*

29. Principal of using Robertson cooked meat broth:
a. Meat kills other bacteria
b. Meat is utilized by anaerobes
c. Content of meat extract utilize O_2
d. All of the above *[Ref. Chakraborty 2/e, p56]*

30. pH of sabouraud's dextrose agar:
a. 7.4
b. 7.0
c. 9.6
d. 5.6
[Ref. Ananthanarayan, 9/e, p 43]

Sabouraud Dextrose Agar
- Culture media for fungi
- Medium has low pH (about 5.0) which inhibits the growth of most bacteria
- Antibacterial agents can also be added to enhance antibacterial effect

31. Medium used for antibiotic senstivity:
a. CLED agar
b. Hektoen agar
c. Mueller-Hinton agar
d. Salt milk agar
[Ref. Ananthanarayan, 9/e, p 635]

Mueller Hinton Agar is most frequently used culture media for antibiotic susceptibility testing.
Read antibiotic sensitivity testing in appendix.

32. Hot air oven is used to sterilize all except:
a. Inoculating loop or wire
b. Glassware
c. Dusting powder
d. Liquid paraffine *[Ref. Ananthanarayan, 9/e, p 30]*

33. Technique of sterilization was introduced by:
a. Robert Koch
b. Edward Jenner
c. Louis Pasteur
d. Lister

34. Inspissation is used for:
a. Sputum
b. Protein containing culture medium
c. Serum containing culture medium
d. Plasma sterilization
[Ref. Ananthanarayan, 9/e, p 30]

35. High level disinfectant are used for:
a. Stethoscopes
b. Electronic thermometers
c. Bronchoscopes
d. Surgical instruments *[Ref. Ananthanarayan, 9/e, p 37]*

36. Hot air over holding time and temperature:
a. 121°C for 15 minutes
b. 160°C for 45 minutes
c. 135°C for 5 minutes
d. 190°C for 30 minutes
[Ref. Ananthanarayan, 9/e, p 30]

Answers **23.** b. Sterilization by... **24.** b. Asbestos filter **25.** a. Phenol **26.** d. All of the above **27.** c. Neisseria
28. a. LJ medium **29.** c. Content of meat extract utilize O_2 **30.** d. 5.6 **31.** c. Mueller-Hinton ..
32. a. Inoculating loop... **33.** a. Robert Koch **34.** b. Protein containing... **35.** c. Bronchoscopes
36. d. 190°C for 30 minutes

CHAPTER

6

Bacterial Genetics

- Molecular genetics begins with central dogma in which DNA (deoxyribonucleic acid) carries genetic information which is transferred to RNA by *transcription* and then *translated* as a particular polypeptide.

$$DNA \rightarrow RNA \rightarrow Polypeptide$$

- This central dogma is seen in all eukaryotes and prokaryotes, however in some virus genetic material is stored in the form of RNA, as in HIV virus.

  Information in this case would be transferred as

$$RNA \xrightarrow[\text{transcription}]{\text{Reverse}} ssDNA \rightarrow dsDNA \rightarrow \text{Integration with host chromosome} \downarrow \text{Viral polypeptide}$$

> I
>
> - **Central dogma:→** DNA → RNA → Polypeptide
> - With few exceptions bacterial genes are hoploid
> - Most prokaryotes contain single circular DNA.

EXTRACHROMOSOMAL GENETIC ELEMENTS

- In addition to chromosomal DNA, most bacteria possess extrachromosomal genetic elements. These are not essential for life but provides additional properties such as drug resistance and toxigenicity
- Plasmids are circular DNA molecules present in the cytoplasm of bacteria capable of autonomous replication. They are seen in some eukaryotes also
- Plasmids encodes properties such as drug resistance, toxin production
- Plasmids can be classified on the basis of:
  a. *Physical methods:* By restriction endonuclease enzyme **(enzyme that cut DNA molecule at or near to specific nucleotide sequence)**. Similar plasmids produce similar restriction endonuclease finger prints
  b. *Genetic methods:* Genetic test can distinguish self transmissible plasmid from non transmissible plasmids. Incompatibility typing is a specific genetic method in which plasmids are classified on the basis of compatibility. Closely related plasmids can not co-exist in the same cell, hence are incompatible.

TRANSMISSION OF GENETIC MATERIAL

Transformation

- Transfer of genetic material through the agency of **free DNA**Q
- Bacteria take DNA from outside, incorporate it with existing DNA through a process called recombination
- Seen in pneumococciQ, *H. influenzae* and certain bacillus species

Transduction

- Transfer of a portion of the DNA from one bacterium to another by a bacteriophage is known as transductionQ
- **MC** mode of gene transfer among prokaryotes
- Transduction happens through either the lytic cycle or lysogenic cycle:
  - ***Lysogenic cycle***: The phage chromosome is integrated (*by covalent bonds*) to the bacterial chromosome where it can remain dormant for thousands of generation. This latent form of phage in which viral genes are present in the bacterium without causing disruption of bacterial cell is called as ***Prophage***.

> I
>
> - **Plasmids:** Extra chromosomal DNA molecules that are capable of autonomous replication. They are not essential for life, but provide some useful powers.
> - **Episome:** Plasmid DNA integrated with chromosomal DNA

- *Lytic cycle*: In lytic or virulent cycle, large number of progeny phages are builtup inside the host bacterium which ruptures (lysis) to release them.

Note: **Generalized transduction**: Transfer of any bacterial gene to another bacterium through bacteriophage
Specialized transduction: Restricted set of bacterial genes is transferred to another bacterium.

I
Genetic Material is transmitted by:
Transformation: First example of genetic exchange to have been discovered. Involve transfer of information via Free DNA
Transduction: Transfer of DNA from one bacterium to other bacterium via_demons, i.e. bacteriophage
Conjugation: Involve contact of two bacterium with one another.

Conjugation

- The process in which donor bacterium makes physical contact with female or recipient bacterium and transfers genetic element into it
- Male status or donor status is determined by the presence of a plasmid which codes for specialised fimbria.
- The plasmid responsible was termed as sex factor or F factor

Genetic Mechanism of Drug Resistance

- Drug resistance may be acquired by mutation or by one of the method of genetic transfer
- Mutational resistance is mainly of two types:
  - *Step wise mutation:* as seen with penicillin where high level of resistance are achieved only by a series of small step mutation
  - *One step mutation:* As seen with streptomycin in which bacteria may become totally resistant to drug after one step mutation

GENETIC BASIS OF DRUG RESISTANCE

Mutation in gene
= Chromosomal mutation

Gene transfer

Transformation
- Significance in nature is not known

Transduction
- Penicillin resistance in staphylococci
- Chromosomal DNA, plasmid and episomes all can be transduced
- Most widespread method of gene transfer

Conjugation
- ***It is most important method of drug resistance.***
- Occurs by plasmid called R factor.
- It can promote chromosomal transfer from diverse bacteria including *Pseudomonas* species

Note:
- Transfer of drug resistance by conjugation of whole plasmid (RTF + r deteminants = R factor) is known as transferable or episomal or infectious drug resistance.
- Enterotoxin and hemolysin production in some enteropathogenic E. coli are also transmitted by RTF (resistance transfer factor).
- Plasmid are genetic elements most frequently transferred by conjugation.

Few facts about HFr Strains

- In conjugation the male status or donor status is determined by presence of plasmid that codes for specialized fimbria. The prototype of this plasmid is called *F factor*
  - Cell containing F factor are called F^+ while lacking ones are called F^-
  - F factor is usually an episome which has got the ability to exist both in free state (as in F^+), as plasmid or integrated with host chromosome (HFR strains). It contains genetic information necessary for synthesis of sex pilus and for self transfer but is devoid of other markers such as drug desistance

I
Prophage: Bacterial genome integrated and inserted into bacterial chromosome or existing as an extrachromosomal plasmid.

Transposons:
Carry information from chromosome to plasmid and vice versa. (Analogous to "Naradh-muni")

- Cells in which the F plasmid is inserted in to chromosome are called HFr strains as they can transfer the gene to recipient cell at a high frequency
- In few HFr strains F plasmid can excise themself in to free state, but during excision they carry some of the neighboring gene. This F-plasmid which has picked up a small chromosome is called ***F-prime (F')***
- This F' when mates with F-transmit some additional gene in addition to F factor. This process resembles transduction and is called ***sex duction.***

Transposable Genetic Elements

- Certain structurally and genetically discrete segment of DNA, move between chromosomal and extrachromosomal DNA molecules within cells. These DNA of molecules are called ***transposons (jumping genes)*** and the process is called transposition.
- Unlike plasmids transposons are not self replicating and depend on chromosomal or plasmid DNA
- They can transfer DNA in molecules that have genetic homology and thus differ from recombination.

Multiple Choice Questions

1. **Virus mediated transfer of host DNA from one cell to another is known as:** [AI 05]
   a. Transduction b. Transformation
   c. Transcription d. Integration

2. **Bacteria may acquire characteristics by all of the following except:** [AI 02]
   a. Taking up soluble DNA fragments across their cell wall from other species
   b. Incorporating part of host DNA
   c. Through bacteriophages
   d. Through conjugation

3. **Lambda phage true ist:** [AIIMS May 11]
   a. Cause mad cow disease
   b. Lytic and lysogenic interconversion can't occur
   c. Lytic form incorporated within host DNA and multiply causing rupture of cell membrane
   d. Lysogenic form incorporates with host DNA and remains dormant

4. **Intraspecies competition is the competition among:**
   a. Species [AIIMS May 05]
   b. Individuals of a population
   c. Individuals of a community
   d. Populations and their regulatory factors

5. **The following phenomenon is responsible for anti-biotic resistance in bacteria due to slime production:**
   a. Co-aggregation [AIIMS Nov 03]
   b. Biofilm formation
   c. Mutation evolving in altered target site for antibiotics
   d. Mutation evolving a target bypass mechanism

6. **Shine-Dalgarno sequence in bacterial mRNA is near:**
   a. AUG codon b. UAA codon [AI 03]
   c. UAG codon d. UGA codon

7. **Plasmid:** [PGI 10, PGI 98]
   a. Involved in multidrug resistace transfer
   b. Involved in conjugation
   c. Imparts capsule formation
   d. Imparts pili formation

8. **True about bacteriophage is:** [AIIMS May 11]
   a. Can transmit toxin to bacteria
   b. Bacteria which transmits DNA to another bacteria
   c. Causes transformation of bacteria
   d. Is a virus which invades bacteria

9. **Which of the following contains both DNA and RNA?**
   a. Plasmids b. Bacteria [AIIMS May 14]
   c. Prions d. Viroids

Explanations and References with Illustrative Answers

1. **Ans. (a) Transduction** *Ref. Ananthanarayan 8/e, p 63-66, 9/e, p 59-60*

Transmission of genetic material = gene transfer = acquisition of characteristic occur by four process.

a. *Transformation:*
   - Transfer of genetic information (about 10-50 genes) through the free DNA.
   - Seen mainly in pneumococci; bacillus species and *Hemophilus influenza.*
   - Any characteristic may be transferred by transformation.

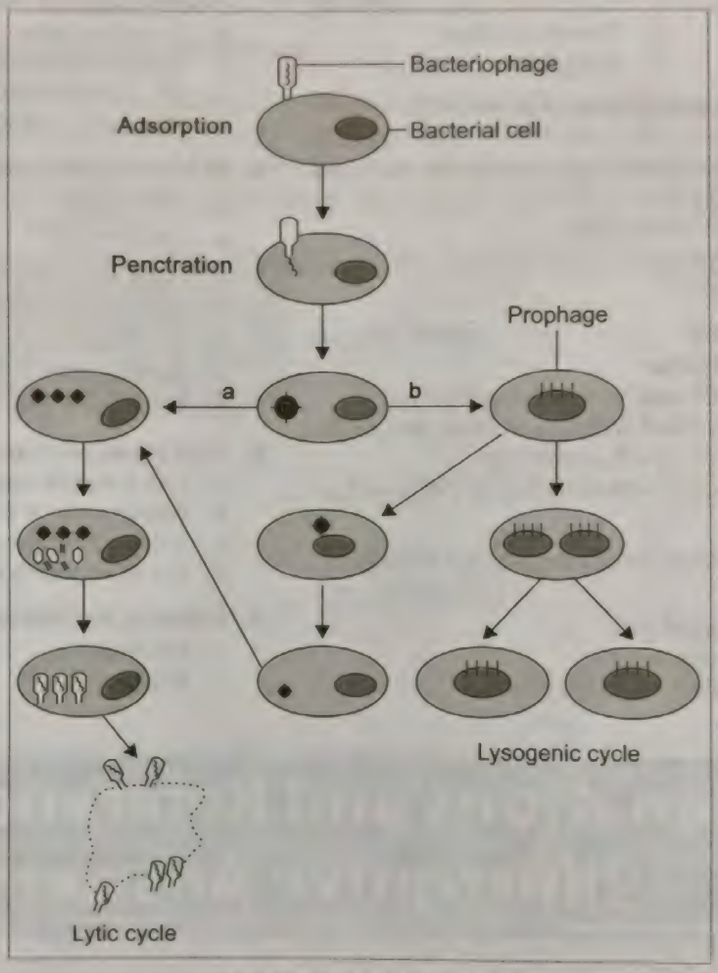
b. *Transduction:*
   - Transfer of portion of DNA from one to other bacteria by agency of bacteriophage (*acts only as vehicle*).
   - It may be generalised (when it involves any segment of donor DNA) or restricted (when specific bacteriophage transduces only a particular genetic trait).
   - It is most widespread mechanism of gene transfer among prokaryotes.
   - Episomes and plasmids (e.g. plasmid determining Pn resistance in staphylococci) may also transduced.
   - It is used in *genetic engineering* in the treatment of some inborn errors of metabolism.

c. *Conjugation*
   - *Bacterial equivalent of sexual mating*
   - Described by Lederberg and Tatum in strain of E. coli (K12), e.g. Transfer of episomes and plasmids of drug resistance.

d. *Lysogenic conversion:*
   - Phage DNA becomes integrated with bacterial chromosome as the prophage which codes for new characteristic, e.g. toxin production in *C. diptheriae.*

2. **Ans. (b) Incorporating part of host DNA** *Ref. Ananthanarayan 8/e p 63; 9/e, p 59-60*
   Already explained

3. **Ans. (d) Lysogenic form incorporates with host DNA and remains dormant** *Ref. Ananthanarayan 8/e, p 456; 9/e, p 60*
   Bacteriophage exhibits two different types of life cycle i.e.
   - Lytic or virulent cycle
   - Temperate or lysogenic cycle



a. Lytic cycle **b.** Lysogenic cycle

a. **Lytic Cycle:** In this cycle bacteriophage enters bacteria, undergo intracellular multiplication, lyse the host bacterum which is followed by release of progeny. This cycle involves following steps:
   - Adsorption
   - Penetration
   - Synthesis of phage components
   - Assembly and maturation of phage particles
   - Lyses of host bacteria
   - Release of progeny

In this cycle phage genetic material never gets incorporated in host bacterial DNA.

Note:
- The interval between the entry of phage nucleic acid into the bacterial cell and appearance of the first infectious intracellular phage particle is known as eclipse phase.
- The interval between the infection of bacterial cell and the first release of infectious phage particle is known as *latent period*

b. Lysogenic Cycle: In this cycle following entry into the host cell. Phage nucleic acid integrates with bacterial chromosome. The integrated phase nucleic acid is known as **prophage** and this behaves like a segment of the host chromosome and replicates synchronomously with it. Lysogeny does not upset the bacterial metabolism, infact it imparts certain new properties.

During the multiplication of lysogenic bacteria some times prophage may get separated and starts lytic cycle *(Hence option b is wrong).*

Note: Lambda phage infects E. coli and imparts it with property of fermenting glucose.

4. Ans. (b) Individuals of a population. *See below*

- *Intraspecies competition:* Competition within a single population
- *Interspecies competition:* Competition between organism of two different species

5. Ans. (b) Biofilm formulation *Ref. Jawetz 24/e, p 158*

- Slime may be polysaccharide (e.g. *Pneumococcus*) or polypeptide (e.g. anthrax)
- A **biofilm** is an aggregate of interactive bacteria attached to solid or to each other and encased in an exopolysaccharide matrix of slime. It forms slimy coat on solid surface and occurs throughout nature.
- A single species or more than one species of bacteria coaggregate to form a biofilm. Fungi including yeasts are occasionally involved
- ***Biofilms make infections persistant and difficult to treat,*** e.g. *S. epidermidis* and *S. aureus* infection of central venous catheters and with *Pseudomonas* airway infection in cystic fibrosis patients.

6. Ans. (a) AUG Codon *Ref. Lippincott 2/e 395*

Learn it.

7. Ans. (a, b, c, d) All options are correct *Ref. Ananthanarayan 9/e p 55, 8/e p 60; Greenwood 18/e, p 75*

Properties encoded by plasmid:

- Resistance to antibiotics (R factor)
- Conjugation by coding for specialized fimbria (sex pilus)
- Bacitracin production, enterotoxin production
- Resistance to toxic metal ions
- Reduced sensitivity to mutagens
- Ability to degrade complex molecules

8. Ans. (d) Is a virus which invades bacteria *Ref. Ananthanarayan 8/e, p 455, 9/e p 456*

Bacteriophage are the virus that infect bacteria. They play on important role in transmission of genetic information between bacteria by the process of ***transduction.***

The presence of phage genome integrated with bacterial chromosome confers certain properties to the bacteria, this process is called ***phage conversion.***

Note:
- Nucleic acid of phage is surrounded by a protein coat
- Some phage also contains lipid
- Most bacteriophage possess ds DNA as genetic material
- In diphtheria bacillus toxin production is determined by the presence of a bacteriophage prophage beta
- So option 'a' is also partially correct, as option 'd' is universal for bacteriophage it is taken as correct answer

9. Ans. (b) i.e., bacteria *See below*

Microbe	DNA	RNA	Protein
Bacteria	+	+	+
Virus	+/–	+/–	+
Prion	–	–	+
Fungi	+	+	+
Parasites	+	+	+
Plasmids	+	–	–
Viroids	–	+	+

Viroids: Plant pathogens that consists of short, strech of circular single/double standed RNA.

Chapter Review

1. **Horizontal transmission of 'R' factor is by:** [Jipmer 03]
   a. Transduction b. Transformation
   c. Conjugation d. Fusion
   [Ref. Ananthanarayan 9/e p 62]

2. **Polymerase chain reaction was discovered by:** [TN 04]
   a. Saiki b. Salk
   c. Watson and Crick d. Mekusick
   [Ref. Robins 7/e 188]

3. **F plasmid of high frequency recombination is :** [APPG 06]
   a. Extrachromosomal b. Chromosomal
   c. Mesosomes d. Ribosomes
   [Ref. Ananthanarayan 9/e p 61]

4. **False regarding bacterial plasmids is?** [Manipal 06]
   a. Extrachromosomal
   b. Eliminated by treating with radiation
   c. Transmission of different characters
   d. Can cause lysogentic conversion
   [Ref. Ananthanarayan 9/e p 54]

5. **Drug resistance transfer by bacteriophage involves:** [MAHE 07]
   a. Transduction b. Conjugation
   c. Transformation d. Convocation
   [Ref. Ananthanarayan 9/e p 59]

6. **The segment of DNA between chromosomal and extrachromosomal DNA molecules within the cells are:**
   a. Transposons b. Cosmid [UP 07, 06]
   c. Plasmid d. Episomes
   [Ref. Ananthanarayan 9/e p 63]

7. **True about transferable resistance:** [Manipal 08]
   a. High degree of resistance
   b. Involves resistance to multiple drugs
   c. Plasmids play a role
   d. All the above *[Ref. Ananthanarayan 9/e p 62]*

8. **Phage typing is useful as an epidemiological tool in all, except:** [JIPMER 2002]
   a. *Salmonella* b. *Staph aureus*
   c. *V. cholerae* d. *Shigella dysenteriae*
   [Ref. Ananthanarayan 9/e p 291]

Salmonella are classified on the basis of distinative O antigen factors

9. **Plasmid:** *[JIPMER 98]*
   a. Involved in multidrug resistace transfer
   b. Involved in conjugation
   c. Imparts capsule formation
   d. Imparts pili formation
   [Ref. Ananthanarayan 9/e p 55]

10. **Following transmit drug resistance except:** [PGI 98]
    a. RTF b. Plasmids
    c. HFr d. Chromosome
    [Ref. Ananthanarayan 9/e p 61]

11. **Drug resistance is not transmitted by:** [PGI 98]
    a. HFr b. Transposons
    c. Plasmids d. Chromosomes
    [Ref. Ananthanarayan 9/e p 61]

12. **Conjugation does not involve:** [PGI 98]
    a. Bacteriophages b. HFr
    c. Fr d. Plasmids
    [Ref. Ananthanarayan 9/e p 59]

13. **Jumping gene is:** [PGI 98]
    a. Transposon b. Episome
    c. Cosmid d. Plasmid
    [Ref. Ananthanarayan 9/e p 63]

14. **Bacterial genome has been completely recognised for which one of the following agents:** [AI 98]
    a. *H. pylori* b. *Yersinia enterocolitis*
    c. *Campylobacter jejuni* d. *Streptococcus*
    [Ref. Harrison 17/e 946]

15. **Microbial killing below MIC is known as:** [DNB 2013]
    a. Adverse effect b. Post antibiotic effect
    c. Both d. None

Post-antibiotic effect

- Defined as the persistant suppression of bacterial growth after a brief exposure of bacteria to an antibiotic even in absence of host defense mechanism.
- Mainly seen with antibiotics that inhibit protein and nucleic acid synthesis. Post antibiotic effect is supposed to be due to alteration of DNA functions.

Answer

1. c. Conjugation	2. d. Mekusick	3. a. Extrachromosomal	4. d. Can cause ...	5. a. Transduction
6. a. Transposons	7. d. All the above	8. a. Salmonella	9. a, b and d	10. c. HFr
11. a. HFr	12. a. Bacteriophages	13. a. Transposon	14. a. H. pylori	15. b. Post antibiotic...

NEET Pattern Questions

1. **Mechanism of direct transfer of free DNA:**
   a. Transformation
   b. Conjugation
   c. Transduction
   d. None

   [Ref. Ananthanarayan, 9/e, p 59]

2. **Transfer of some chromosomal material from one bacteria to other can occur via:**
   a. F factor
   b. R factor
   c. Transfer factor
   d. All of the above

   [Ref. Ananthanarayan, 8/e, p 65; 9/e, p 61-62]

3. **R-factor in bacteria is transferred by:**
   a. Transduction
   b. Transformation
   c. Conjugation
   d. Vertical transmission

4. **Multiple drug resistance is spread by:**
   a. Transformation
   b. Transduction
   c. Mutation
   d. Conjugation

Multiple drug resistance is transferred by R plasmid through conjugation.

5. **Viral DNA is integrated into Bacterial DNA in:**
   a. Transduction
   b. Lysogenic conversion
   c. Transformation
   d. Conjugation

   [Ref. Ananthanarayan 9/e, p 60]

6. **Prophage is defined as:**
   a. Insertion of viral nucleic acid into bacteria by bacteriophage
   b. First cycle of division of bacterial nucleic acid
   c. Last cycle of division of bacterial nucleic acid
   d. Integrated temperate bacteriophage genome into bacterial chromosome

Answers 1. **a.** Transformation 2. **d.** All of the above 3. **b.** Transformation 4. **d.** Conjugation
5. **b.** Lysogenic conversion 6. **a.** Insertion of viral nucleic acid...

Section - B

UNIT – I Bacteriology

Gram-positive Cocci

- Staphylococci
- Streptococci

Gram-negative Cocci

- Neisseria

Gram-positive Bacilli

- Clostridium
- Corynebacterium
- Actinomycetes and Bacillus
- Listeria Monocytogenes
- Mycobacteria

Gram-negative Baccilli

- Enterobacteriaceae
- Vibrio
- Pseudomonas and Yersinia

Gram-negative Bacilli and Cocco-bacilli

- Hemophilus, Bordetella and Brucella
- Campylobacter and Helicobacter
- Legionella
- Rickettsiae and Chlamydiae

Others

- Spirochetes
- Mycoplasma

CHAPTER

7

Staphylococci

Catalase positive, nonmotile, aerobic and facultatively anerobic organism, that do not form spores

Morphology:
Staphylococci are spherical cells arranged in irregular clusters. Young cocci stain strongly Gram positive. On ageing, many cells become gram negative.

Culture:
Staphylococci grow readily on most media. They grow most rapidly at 37°C but pigment production is maximum at room temperature (20-25°C). **S. aureus** forms grey to deep golden yellow colonies. **S. epidermidis** forms gray to white colonies. Under anaerobic condition there is no pigment production.

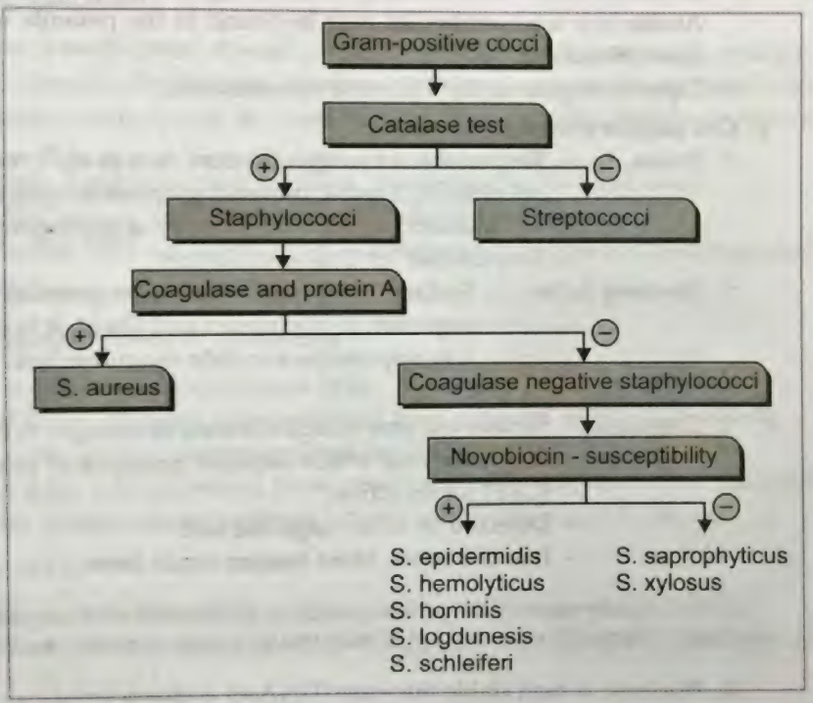
I

Gram-Positive Cocci:

- Staphylococci
- Streptococci
- Pneumococci

} Catalse (–)ve

CLASSIFICATION



Classification of Staphylococci	
Coagulase+ve / Protein A+ve	**Coagulase–ve**
• More virulent	• Less virulent
• Form golden yellow colonies on solid media	• Form white colonies
• Usually pathogenic	• Usually not pathogenic

I

Site of colonization of S. aureus

- Anterior nares
- Axillae
- Perineum
- Vagina

STAPHYLOCOCCI AUREUS

- Part of normal human flora; 20-25% of healthy individuals may be persistantly or transiently colorized. Anterior nares are the frequent site of human colonization.
- The colonization sites serve as a reservoir of strains for future infection and persons colonized with S. aureus are at higher risk for infection.
- *MC* route of infection – Skin.
- ***MC* source of infection:** Patients own colonizing strain. *... Harrison 19/e, p955*

Culture

- On nutrient agar, show characteristic *oil paint appearance.*
- *Show β hemolysis which is marked on rabbit or sheep blood and weak on horse blood agar.*
- Salt milk agar, salt broth and *ludlam's medium are selective medium* ...*AA 9/e p 200*

Biochemical Reaction

- **Mannitol fermenter** anaerobically *(not by other species of Staphylococci)*
- **Phosphatase reaction**: – Gives prompt phosphatase reaction.
  – Useful screening procedure as *S.epidermidis is usually negative or only weakly positive.*

I

Distinctive features of S. aureus:
- Coagulase
- Thermostable nuclease
- Clumping factor
- Shows β hemolysis

Source of infection
- Direct contact is the most important mode
- Airborne
- Through fomites

Virulence Factors

- Most constant association of *virulence* is production of enzyme *coagulase* and to a lesser extent with *mannitol fermentation.*

a. Cell associated polymers:
- *Cell wall polysaccharide peptidoglycan*: Activates complement system. Induce release of cytokines, possess endotoxin like activity.
- *Techoic acid:* For adhesion and protection against complement mediated opsonization. Antitechoic acid antibodies may be found in the patients with active endocarditis due to S. aureus.
- *Capsular polysaccharide*: Decrease opsonization.

b. Cell surface proteins:
- *Protein A:* – Responsible for coagglutination. Acts as an Fc receptor. Binds to Fc terminal of IgG 1, 2 and 4, preventing opsonophagocytosis by neutrophils.
  – Chemotactic, anticomplementary, antiphagocytic and B-cell mitogen.
  – Responsible for Coagglutination.
- *Clumping factor:* – Surface compound that is responsible for adherance of the organism to fibrinogen and fibrin. It is distinct from coagulase. It is responsible for slide coagulase test. ... *Jawetz 25/e, p 187*

c. Extracellular enzymes

1. *Coagulase:* – Surface enzyme which converts fibrinogen to fibrin.
   – It is a enzyme which requires presence of coagulase releasing factor (CRF) for its action.
   – Detected by tube coagulase test.
   – It is of 8 types. Most human strain form type - A coagulase.

Caution: Initially clumping factor is supposed to be bound form of coagulase. Now it is clear that it is totally different. So, the concept of slide test for bound cogulase has diminished.

2. *Nuclease:* A heat stable nuclease (DNAse) is **characteristic** of *S.aureus*
3. *Protein receptors:* For mammalian proteins e.g. fibronectin, fibrinogen, IgG, C1q. Facilitates adhesion.
4. *Lipases:* Helps in infecting skin and subcutaneous tissue
5. Hyaluronidase.
6. Protease
7. Fibrinolysin (Staphylokinase) ┐ Helps in spread of infection

Toxin

A. Cytolytic Toxins:

- α *Hemolysin:* – Most important hemolysin
  – Protein inactive at 70°C, but reactivated paradoxically at 100°C.
  – Lyses rabbit erythrocyte but is less active against human erythrocytes.
  – Leukocidal, cytotoxic, dermonecrotic, neurotoxic and lethal.
- β Hemolysin – Shows 'hot cold phenomenon'. Sphingomyelinase, hemolytic for sheep cells.

- γ Hemolysin — Bicomponent protein.
- δ Hemolysin — Detergent like effect on cell membranes, plays role in S. aureus diarrheal disease
- Leukocidin (Panton valentine Toxin) — Bi component toxin associated with farunculosis. Important virulence factor in MRSA infection.

...Jawetz, 27/e, p 206

Note: *Synergohymenotropic toxin:* Bi component toxin such as Leukocidin and γ Hemolysin.

I

Toxins:

Staphylococcal enterotoxin: Heat stable enterotoxin
TSST: Superantigen
Leukocidin & γ Hemolysin: Bicomponent toxin (synergohymenotropic toxin)

B. *Enterotoxin:* (A, B, C_{1-3}, D, E and H)

- Preformed, heat stable toxin, responsible for staphylococcal food poisoning which occur 2-6 hrs after consuming meat and fish, milk or milk products.
- *Source:* Usually food handler which is carrier.
- *Mechanism:* Toxin acts directly on autonomic nervous system *(Vagal stimulation)* and vomiting center.
- *Type A* toxin is responsible for *most* cases.

C. *Toxic shock syndrome toxin (TSST)*

- Toxic shock syndrome is multisystem disease presenting with fever, hypotension, myalgia, vomiting, diarrhea, mucosal hyperemia, erythematous sunburn, rash, disorientation or altered consciousness seen mostly in menstruating women using highly absorbent vaginal tampons.
  - TSST-1 = Enterotoxin F = Pyrogenic Exotoxin C is responsible for most cases.
  - *TSST-1 binds to MHC class II molecules, yielding T-Cell stimulation.*
  - Staph. Enterotoxin and TSST are *super antigen* leading to an excessive and non regulated immune response.

D. *Exfoliative / Epidermatolytic Toxin / ET / Exfoliatin*

- Cause staphylococcal scalded skin syndrome (SSS).
- Severe form is called Ritter's disease *in* ***neonate*** and *toxic epidermal necrolysis* in ***elderly.***
- Milder form are pemphigus neonatorum and bullous impetigo.
- There are two type: **ETA** (chromosomal gene product, heat stable) and **ETB** (plasmid mediated, heat labile). Possess serine protease activity which triggers exfoliation.

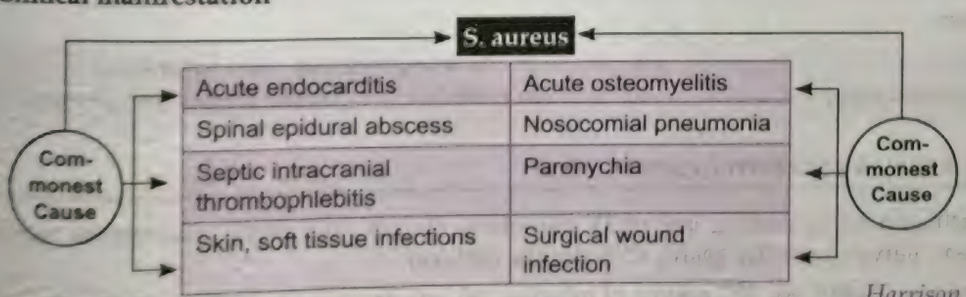
Typing

- Staphylococci are typed on the basis of their susceptibility to bacteriophage.
- Phage typing is done by pattern method, Employing 1 over 28 phages. Most hospital epidemics are caused by group I.

I

Toxic shock syndrome toxin. Mostly caused by TSST-1 = Pyrogenic exotoxin C.

Clinical manifestation



....Harrison 19/e, p 955

- *S.aureus is* ***MC*** *cause of acute endocarditis except early and late prosthetic valve endocarditis which are caused by Coagulase-ve staph. and Streptococci viridans respectively.*
- Though rare, recently methicillin resistant S. aureus (MRSA) have been reported as primary cause of community acquired pneumonia. .... Harrison 19/e, p 955

Remember: Subacute endocarditis is typically caused by Strep. *viridans.*

I
No antibotic treatment is recommended in staphylococcal food poisoning.

Disease Casued by Staph. aureus

Skin and Soft Tissue Infections
–Folliculitis –Furuncle, –Carbuncle –Cellulitis
–Impetigo –Mastitis –*Surgical wound infections* –Hidradenitis suppurativa

Musculoskeletal Infections
–Septic arthritis –Osteomyelitis –Pyomyositis –Psoas abscess

Respiratory Tract Infections
–Ventilator-associated or nosocomial pneumonia –Septic pulmonary emboli
–Postviral pneumonia (e.g., influenza) –Empyema

Bacteremia and Its Complications
–Sepsis, septic shock –Metastatic foci of infection (kidney, joints, bone, lung)

Infective Endocarditis

Device-Related Infections (e.g., intravascular catheters, prosthetic joints)

–*Toxin-Mediated Illnesses*
–Toxic shock syndrome –Food poisoning
–Staphylococcal scalded-skin syndrome

Invasive Infections Associated with Community-Acquired MRSA
–Necrotizing fasciitis –Waterhouse-Friderichsen syndrome
–Necrotizing pneumonia

Lab diagnosis

- Diagnosis is made by *culture,* specimen is plated on blood agar.
- Smears are examined from culture and coagulase test is done.
- Coagulase test is the standard criterion for S. aureus identification. It is done by two methods - tube and slide coagulase test. Slide coagulase test is simpler while tube coagulase test is more specific.
- *Serological Test*: – Helpful in diagnosis of hidden deep infection.
  – Antistaphylolysin (antialphalysin) titre of more than two unit is important specially, when rising.
- Polymerase chain reaction (PCR) based assays have been applied for rapid diagnosis of S.aureus infection.

... Harrison 19/e, p 956

Treatment

- If sensitive to penicillin → Penicillin G
- Penicillinase producing but sensitive to methicillin → Naficillin or Oxacillin
- Methicillin resistant Staph. aureus (MRSA) → Vancomycin
- Vancomycin resistant Staph. aureus (VRSA) → Quinopristin, dalfopristin, linezolid
- Emperical therapy → Vancomycin

Special Cases

- TSS → Clindamycin (reduces toxin synthesis)
- Food poisioning → *No antibiotic* (as caused by preformed toxin)

COAGULASE (–) VE STAPHYLOCOCCI (CONS)

- ***MC*** pathogen complicating use of IV catheters, shunts and grafts, pacemaker wires, prosthetic valves, vascular grafts, CSF shunts, dialyser.

Mnemonic—CoNS are ***MC*** source of infection on any exogenous implant.

I
(Cogulase (–) ve staphylococci
– Staph. epidermidis (MC)
– S. Saprophyticus (UTI)
– S.lugdunensis (severe)

Staph epidermidis / albus

- Normally present on human skin. Not pathogenic ordinarily.
- Predilection for growth on implanted foreign bodies.
- Common source of stich abscess.

- *S. epidermidis* is adapted to colonize these devices by its capacity to elaborate the extracellular polysaccharide (glycocalyx or slime) that facilitates formation of protective biofilm on the device surface. This biofilm protects bacteria from antibiotics and host defence.
- The attachment is also facilitated by autolysis (AtlE), fibrinogen binding protein, and accumulation-associated protein (AAP).

Staph. saprophyticus

- Non-pigmented, novobiocin resistant, and non hemolytic
- Present on normal human skin and periurethral area.
- Cause UTI in sexually active young women. This is due to its enhanced capacity to adhere to uroepithelial cells.

....Jawetz 27/e p 206

S. lugdunensis* and *S. schleiferi

- Produces serious infections (native valve endocarditis and osteomyelitis) than do other CoNS.

Other CoNS are:

- *S. xylosus* , • *S. hominus* , • *S. haemolyticus*

Micrococci

- Gram positive, catalase positive, oxidase positive aerobic bacteria that resemble staphylococci.
- Differentiated from staphylococci through Hugh and Leifson oxidation fermantation test.

Multiple Choice Questions

1. **A diabetic patient developed cellulitis due to *S.aureus*, which was found to be methicillin resistant on the antibiotic sensitivity testing. All the following antibiotics will be appropriate except:** [AI 06]
   a. Vancomycin b. Imipenem
   c. Teicloplanin d. Linezolid

2. ***Staphylococcus aureus* differs from staphylococcus epidermidis by:** [AI 02]
   a. Is coagulase positive
   b. Forms white colonies
   c. A common cause of UTI
   d. Causes endocarditis of prosthetic valve

3. **Which of the following statement is most correct regarding resistance to methicillin in MRSA:**
   a. Resistance is produced as a result of alteration in penicillin binding protein [AI 2011]
   b. Resistance is produced by production of β lactamase
   c. Resistance is mediated by plasmids
   d. Expression of resistance is enhanced by incubating at 38°C during susceptibility testing

4. **All of the following statement are true about Staphylococci except:** [AIIMS 04]
   a. A majority of infection caused by coagulase (-) ve Staph. are due to *staph. epidermidis*
   b. b-Lactamase production is under plasmid control
   c. Expression of methicillin resistance in *Staphylococcus aureus* increases when it is incubated at 37°C on blood agar
   d. Methicillin resistance in *Staph. aureus* is independent of b-Lactamase production

5. **Which one of the following Gram positive organism is most common cause of UTI among sexually active women:** [AIIMS 04]
   a. *Staphylococcus epidermidis*
   b. *Staphylococcus aureus*
   c. *Staphylococcus saprophyticus*
   d. Enterococcus

6. **The following is characteristic feature of *staphylococcus* food poisoning except:** [AIIMS 04]
   a. Optimum temperature for toxin production is 37°C
   b. Intradietic toxin are responsible for intestinal symptoms
   c. Toxin can be destroyed by boiling for 30 minutes
   d. Incubation period is 1-6 hours

7. **A patient in an ICCU is on CVP line. His blood culture shows growth of Gram-positive cocci which are catalase positive and coagulase negative. The most likely etiological agent is:** [AIIMS 03]
   a. *Staph. aureus*
   b. *Staph. epidermidis*
   c. *Streptococcus pyogenes*
   d. *Enterococcus faecalis*

8. ***Staph. aureus* causes vomiting in 6-8 hours. The mechanism of action is:** [AIIMS 02]
   a. Stimulation of CAMP
   b. Vagal stimulation
   c. Stimulation of CGMP
   d. Acts through ganglioside GM receptor

9. **A cook prepares sandwiches for 10 people going for picnic. Eight out of them develop severe gastroenteritis within 4-6 hrs of consumption of the sandwiches. It is likely that on investigations the cook is found to be carrier of:** [AIIMS 02]
   a. *Salmonella typhi* b. *Vibrio cholerae*
   c. *Entamoeba histolytica* d. *Staphylococcus aureus*

10. **A child after consuming food in a party complains of diarrhea within 1-5 hours. The diagnosis is:**
    a. *S. aureus* [AIIMS 01, 96, 95]
    b. *Streptococcus*
    c. *Clostridium perfringens*
    d. *Clostridium botulinum*

11. **Synergohymenotropic toxin of staphylococci consist of:** [PGI May 2011]
    a. α toxin b. β toxin
    c. γ toxin d. δ toxin
    e. Panton valentine toxin

12. **Which of the following organisms is implicated in the causation of botryomycosis:** [PGI 01]
    a. *Staphylococcus aureus*
    b. *Staphylococcus albus*
    c. *Pseudomonas aeruginosa*
    d. *Streptococcus pneumonia*
    e. *Streptococcus pyogenes*

13. **Staphylococcus in stool occurs in:** [PGI 01, 00]
    a. Staphylococcal food poisoning
    b. Ischiorectal abscess
    c. Toxic shock syndrome
    d. May be a normal finding
    e. Pseudomembranous colitis

14. ***Staphyloccus* can cause:** [PGI 01]
    a. Ecthyma
    b. Erythrasma
    c. Furuncle
    d. Impetigo contagiosa
    e. Sycosis barbae

15. **A 25-year-old IV drug abuser presents with fever for 3 weeks. ECHO shows tricuspid vegetation. The most likely organism associated with endocarditis in this case is:** [AIIMS Nov 09, May 11]
    a. *Staph. aureus*
    b. *Candida albicans*
    c. *Pseudomonas*
    d. *Strep. viridans*

16. **All of the following statements about staphylococcus aureus are true: except** [AI 2010]
    a. Most common source of infection is cross infection from infected people
    b. About 30% of general population is healthy nasal carriers
    c. Epidermolysin and TSS toxin are superantigens
    d. Methicilin Resistance is chromosomally mediated

Explanations and References with Illustrative Answers

1. **Ans. (b) Imipenem** *Ref. Harrison 18/e, p1168, 19/e, p962; Katzung 9/e, p768*
"Imipenem is not effective against Enterococcus faecium, MRSA, Clostridium difficile, Burkholderia cepacia as they produce metallobetalactamases." Though it is effective against methicillin sensitive staph. aureus.

Treatment of MRSA		
Drug of choice	**Alternative**	**Investigatory**
Vancomycin	TMP - SMX	Oritavancin
	Minocycline	Tigecycline
	Ciprofloxacine, Levofloxacin	Ceftobipirole
	Quinupristin/dalfopristin	
	Linezolid	
	Daptomycin	
	Ceftaroline	

Antibiotic Resistance		
Agent	**Mechanism of resistance**	**Site**
Penicillins	β-lactamase	Plasmid
Methicilin, Cephalosporin	Altered binding protein	Chromosome
Chloramphenicol	Acetyl transferase	Plasmid
Erythromycin	Methylation of ribosome	Plasmid
Streptomycin	Altered ribosomal protein	Chromosome
Vancomycin	Van A gene	Chromosome
Quinolones	Mutation in topoisomerase IV	

Note: Mechanism of vancomycin resistance involves the replacement of the last D-alanine residue of peptidoglycan precursors with D-lactate or D-serine resulting respectively in high level and low level resistance. *Harrison 19/e p 1187*

Remember:
- Ceftaroline: A novel cephalosporin with activity against MRSA ... *CMDT 2014, p 1378*
- Adjuvant drugs for MRSA include gentamycin, rifampicin, fusidic acid
- Teicloplanin is glycopeptide which is similar to vancomycin in both mechanism and spectrum of activity.
- Drugs for **VRSA (vancomycin resistant S.aureus):**
  – Daptomycin / dalfopristin – Quinupristin – Linezolid. – TMP – SMX

Note: S. aureus is a member of ESKAPE; group of resistant bacterias which includes. Enterococcus faecium, S. aureus, Klebsiella sp, Acinetobacter baumannii, Pseudomonas aeruginosa, and enterobacter sp

2. **Ans. (a) Is coagulase positive** *Ref. Ananthanarayan 8/e, p202*

Property	Staphylococcus aureus	Staphylococus epidermidis
Coagulase	Positive	Negative
Mannitol	Ferments	Not ferment
Pathogenicity	Pathogenic	Less pathogenic
Colony	Golden	White
Hemolysis	Show	Not show
Cause Endocarditis	In normal native valve	In prosthetic valve

3. **Ans. (a) Resistance is produced** *Ref. Katzung 10/e, p727; Harrison 19/e, p961*

"Production of novel penicillin binding protein (PBP 2a or 2') is responsible for methicillin resistance". Protein is synthesized by mec A gene which is part of a large mobile genetic element called ***SCC mec***.

It is hypothesized that the genetic material was acquired via horizontal transfer from a related species, S. sciuri.

Drug resistance in staphylococcus

(A) Penicillin resistance:

1. Production of β lactamase:
   - Plasmid mediated inducible enzyme which is transmitted by transduction (more commonly) or conjugation. Now only <5% of strains of staph are sensitive to penicillin.
   - Hospital strains mostly form type A penicillinase.
   - Same plasmid carry genes for resistance to tetracycline, erythromycin, aminoglycoside too.
2. Altered penicillin binding protein:
   - Due to production of novel penicillin binding protein 'PBP2a', this protein is synthesized by mecAgene.
   - Chromosomally mediated, expressed more at 30°C than at 37°C.
   - Responsible for resistance against penicillinase resistant penicillin such as methicilin, cloxacillin. Strains are called **MRSA** (Methicillin resistant Staph. aureus).
   - MRSA escape methicillin by synthesizing an extra PBP called PBP-2A which carries out function of those PBP's that are inactivated by methicillin.
3. *Tolerance to pencillin:*
   - Bacteria only inhibited not killed.

(B) Vancomycin

1. *Intermediate susceptible strain:* Also known as **VISA** (*vancomycin-intermediate S. aureus*). For these VISA strain **MIC** (*minimal inhibitory concentration*) of vancomycin is 4-8 μg/ml. The mechanism of resistance is increased cell wall synthesis and alteration in the cell wall.
2. *Resistant strain:* Also known as **VRSA** (*vancomycin resistant strain*): This is due to presence of van A gene

(C) Others

- Plasmid mediated resistance to teracycline, erythromycin, aminoslycosides

4. **Ans. (c) Expression of methicillin resistance in *Staphylococcus aureus* increases when it is incubated at 37°C on blood agar** *Ref. Ananthanarayan 8/e, p197, 9/e, p201; Jawetz, 25/e p 186*

"Methicillin resistance is expressed more when Staph. is incubated at 30°C than at 37°C."

Refer Ans. No. 3 for full explanation.

5. **Ans. (c) *Staphylococcus Saprophyticus*** *Ref. Harrison 19/e, p960, 18/e, p1166; Ananthanarayan 8/e, p202, 9/e, p206*

S. saprophyticus cause UTI in young women due to its enhanced capacity to adhere to uroepithelial cells. A 160 KDa hemagglutinin adhesins may contribute to this affinity.

Note: S. Saprophyticus is novobiocin resistant.

6. **Ans. (c) Toxin can be destroyed by boiling for 30 minutes** *Ref. Harrison 19/e, p959, 18/e, p1165*

Staphylococcal food poisoning:

- Staphylococcal food poisoning is due to *heat stable preformed toxin* mostly after consuming milk products.
- Toxin is produced optimally at 35°C to 37°C and is resistant to the action of gut enzymes
- As disease is due to toxin, secondary spread is not there
- Main d/d is B. cereus food poisoning.
- *IP*- 1-6 hours.
- *Mechanism* - Acts by **stimulating vagus nerve and vomiting center of brain**
- *No antibiotics* are given for staphylococcal food posioning.
- Treatment is conservative.

7. **Ans. (b) Staph. epidermidis** *Ref. Harrison 19/e, p954, 960; 18/e p 1166*

Gram +ve, catalase +ve, coagulase -ve bacteria is **Coagulase-negative *Staph.***

S. epidermidis is uniquely adapted to colonize prothestic devices due to its capacity to elaborate the extracellular polysaccharide ***(glycocalyx or slime)*** that facilitates formation of protective biofilm on the device surface. This slime production also protect the bacteria against antibiotics.

Cogulase (–)ve *Staphylococcus*	
S. epidermidis	**S. saprophyticus**
• Novobiocin sensitive	• Novobiocin resistant
• Predilection for growth on implanted foreign bodies	• Not so
• *Most common* cause of *prosthetic valve endocarditis*	• Cause *UTI in sexually active young women*

Remember: Both are present on normal skin and are not usually pathogenic.

8. **Ans. (b) Vagal stimulation** *Ref. Harrison 17/e, p875, 18/e p 1163*

Following line from ***Harrison*** makes the answer clear to you.

"Staph. food poisoning result from heat stable preformed enterotoxin. Due to preformed nature incubation period is short. Toxin stimulates the vagus nerve and vomiting center of brain. It also appears to stimulate intestinal persistaltic activity".

9. **Ans. (d) Staphylococcus aureus** *Ref. Harrison 17/e, p816, 18/e p 1087. 19/e p 266*

Learn this table by heart, every year there is question on food posioning.

Bacterial food posioning		
Incubation Period, Organism	**Symptoms**	**Common food source**
1 to 6 H		
Staphylococcus aureus	Nausea, vomiting, diarrhea	Ham, poultry, potato or egg, salad, mayonniase, cream pastries
Bacillus cereus	Nausea, vomiting	Fried rice
8 to 16 H		
Clostridium perfringens	Abdominal cramps, diarrhea *(vomiting rare)*	Beef, poultry, legumes, gravies
B. cereus	Abdominal cramps, diarrhea vomiting	Meats, vegetables, dried beans, cereals
>16 H		
Vibrio cholerae	Watery diarrhea	Shellfish
Enterotoxigenic *Escherichia coli*	Watery diarrhea	Salads, cheese, meats
Enterohemorrhagic *E. coli*	Bloody diarrhea	Ground beef, raw vegetables
Salmonella spp.	*Inflammatory diarrhea*	Beef, poultry, eggs, dairy products
Campylobacter jejuni	*Inflammatory diarrhea*	Poultry, raw milk
Shigella spp.	*Dysentery*	Potato or egg salad, lettuce, raw vegetables
Vibrio parahaemolyticus	Dysentery	Mollusks, crustaceans

- S. aureus is ***MC*** cause of food poisoning in west.

10. **Ans. (a) S. aureus** *Ref. Harrison 17/e, p816, 18/e, p 1165, 19/e p 266*

Incubation period in food poisoning of S.aureus is 1-6 hours.

11. **Ans. (c) and (e) i.e. γ toxin and Panton - Valentine toxin** *Ref. Ananthanarayan 8/e, p198, 9/e, p 202*

Synergohymenotropic toxins are a family of bicomponent toxin that acts through the synergistic action of two non-associated secretory proteins. *Staphylococcal leucocidin (Panton-Valentine toxin) and gamma hemolysin are the example of synergohymenotropic toxins.*

12. **Ans. (a) *Staphylococcus aureus*** *Ref. Ananthanarayan 8/e, p393, 9/e, p 394*

Botyromycosis is a chronic granulomatous condition similar to mycetoma, usually involves the skin and characterized by granules in the pus, consisting of masses of bacteria generally *Staphylococcus aureus.*

13. Ans. is (a) Staphylococcal food poisoning *Ref. Ananthanarayan 8/e, p198, 9/e, p204*

- In case of staphylococcal food poisoning food remnant and faeces are inoculated on ***selective medium*** like **ludlam's or salt milk agar** or **Roberson cooked meat medium** *containing 10% NaCl.*
- TSS is due to systemic effect of absorbed toxin from site such as vagina, so there is no possibility of finding Staph. in case of TSS.
- Ischiorectal abscess is mostly due to *E. coli.*
- Normal intestinal flora usually don't contain *Staph. aureus.* So it is *not* a normal finding.
- Pseudomembranous colitis is caused by *Clostridium difficle.*

14. Ans. (c) (d) and (e) Faruncle, Impetigo contagiosa and Sycobis barbae *Ref. Harrison 18/e, p 1163;, 19/e p 956 CMDT 2014, p 126*

Impetigo contagiosa is caused by S. aureus or Streptococcus or both. It is the most common type of impetigo.

Skin and soft tissue infection of *S. aureus*

- ***Folliculitis:*** Superficial infection of ostia of hair follicle.
- ***Faruncles (boils):*** More extensive painful lesions that tends to occur in hairy moist region of body and extend from hair follicle to become a true abscess, e.g. buttock.
- ***Carbuncle:*** Mostly located in lower neck and are even more severe and painful.
- ***Acute paronychia: MC*** cause is *S.aureus.*
- ***Bullous impetigo:*** Impetigo is *most frequently diagnosed bacterial infection. Almost always caused by S. aureus.*
- ***Ecthyma:*** *It is deeper form of impetigo caused by Staph or Strep.*
- ***Cellulitis***
- ***Hiradenitis suppurativa:*** Recurrent follicular infections in region such as axilla.
- **Sycosis barbae** is *chronic folliculitis of beard hair follicle.*

Remember:

- S. aureus is most common cause of surgical wound infection and is second only to CoNS as a leading cause of primary bactremia. ..... Harrison 19/e, p 955
- Non-bullous impetigo, cellulitis and erysipelas is caused by Strep. pyogenes more commonly than S.aureus.
- Ecthyma gangrenosum is caused by Ps. aeruginosa.
- Erythma migrans is caused by Borrelia burgdorferi (Tick transmission).
- Erythrasma is caused by corynebacterium minutissimum.
- Impetigo contagiosa is usually caused by streptococci or mixed infections.
  - Most cases of impetigo are caused by S.aureus.

15. Ans. (a) *Staph. aureus* *Ref. Harrison 17/e p 789,790*

Endocarditis in IV drugs addicts	
Right sided (more common)	**Left sided**
Staph. aureus	Varied (Staph, aureus, enterobacter, polymicrobial)

16. Ans. (a) Most common source of infection... *Ref. Jawetz 27/e p 171 ; Harrison 18/e, p 1161, 19/e, p 955*

Let consider each option

Option 'a', 'b'

Epidemiology of Staph aureus

- Part of normal flora, nasal carriage occurs in 20-25% of humans. *Rate of colonization is higher among HIV patients, insulin dependent diabetes, patients undergoing hemodialysis, individuals with skin damage.* ***Anterior nares*** are the ***most common*** site of colonization; although skin, axilla, vagina, perinium may be colonized.
- Most individual who develop S. aureus are infected with their own colonizing strain. Contact spread of infection is more common in hospitals, where transmission most frequently occurs through hands of hospital personel. *Heavily colonized individual can transmit bacteria via aerosols of respiratory or nasal secretions.*

Option 'c'

- Toxic shock syndrome toxin (TSST-1), epidermatolytic toxin and other staphylococcal enterotoxin are superantigens.
- These superantigens can bind MHC molecule outside the peptide binding cleft. Consquently super antigen can activate upto 10% of T-cell in nonspecific manner which in turn leads to release of large quantity of cytokines.

Option 'd'

- Resistance to methicillin (and nafcillin) is encoded and regulated by a sequence of genes located in a region of chromosome called ***staphylococcal cassette chromosme mec*** (*SCC mec*). This gene encodes for a low affinity penicilin binding protein.

Chapter Review

1 Tropical pyomyositis is caused by: [AP 2007]
a. Streptococcus
b. Staphylococcus aureus
c. Pseudomonas
d. Pneumococcus
[Ref. Harrison 19/e, p958, 18/e, p1068]

2. The antibody marker in serum for staphylococal endocarditis is: [AIIMS 90]
a. ASLO
b. Antitechoic acid
c. Anti lipopolysaccharide
d. Anti-M-protein
[Ref. Ananthanarayan 8/e, p197, 9/e, p201]

3. Methicillin resistance in Staph. aureus is due to: [AP 2007]
a. β-lactamase
b. MECA gene
c. AMPC gene
d. Porin develop
[Ref. Jawetz 27/e 204]

MeCA gene codes for altered penicillin binding protein which is not affected by methicillin, oxacillin and nafcillin.

4. Pyomyositis is caused by : [Delhi 2008]
a. Clostridium
b. Staph. aureus
c. Streptococcus
d. E. coli
[Ref. Harrison 19/e, 958, 18/e, p1068]

Pyomyositis is usually caused by S. aureus while primary myositis is usually caused by Streptococcus pyogenes.

5. Acute haematogenous osteomyelitis is commonly caused by :
a. Staph. aureus
b. Strepto. pneumoniae
c. E. coli
d. Pneumococcus
[Ref. Harrison 19/e, p957]

6. Staphylococcus aureus remains in the skin for: longer period because of: [MAHE 01]
a. Catalase
b. Coagulase
c. Hyaluronidase
d. None *[Ref. Ananthanarayan 8/e, p198, 9/e, p202]*

7. Postoperative parotitis is caused by: [Delhi 00]
a. Staph aureus
b. Streptococcus
c. E. coli
d. Pneumococcus
[Ref. CSDT '12/e, p28]

8. Mohan comes from dinner he compain of diarrhea, vomiting after 4 hours of meal. Most likely causative agent: [UP 02]
a. Stap aureus
b. V. cholerae
c. Streptococcus
d. E. coli
[Ananthanarayan 8/e, p198, 9/e, p202]

9. Quikest food poisoning (1-6) hour is: [Kolkata 02]
a. Staphylococcus
b. B. cereus
c. Salmonella
d. Vibrio cholera
[Ref. Harrison 19/e, p959, 18/e, p1088]

10. Cutaneous mainfestation of staphylococci are all except: [St Johns 02]
a. Faruncle
b. Follicular impetigo
c. TSS
d. TEN
[Ref. Ananthanarayan 8/e, p199, 9/e, p203]

11. Toxin of staphylococcus: [Bihar 03]
a. Hemolysin
b. Leukocidin
c. Enterotoxins
d. All
[Ref. Ananthanarayan 8/e, p198, 9/e, p202]

12. Bacterial endocarditis is most commonly caused by:
a. Alpha haemolytic streptococci [DNB 2013]
b. Beta haemolytic streptococci
c. Staphylococcus aureus
d. None
[Ref. Harrison 18/e, p1052, 1165]

13. Staphylococcus secrete all, except: [Bihar 04]
a. Lipase
b. Cellulose
c. Coagulase
d. Lecithinase
[Ref. Ananthanarayan 8/e, p198, 9/e, p202]

14. The most common mechanism of drug resistance in staphylococcus is: [M.P. 04]
a. Conjugation
b. Plasmids
c. Transduction
d. Translation
[Ref. Ananthanarayan 8/e, p197, 9/e, p201]

Answers 1. b. *Staphylo..* 2. b. Antitechoic ... 3. b. MECA... 4. b. Staph... 5. a. *Staph. aureus*
6. c. Hyaluronidase 7. a. *Staph aureus* 8. a. *Stap aureus* 9. a. *Staphylococcus* 10. c. TSS
11. d. All 12. c. *Staphylococcus...* 13. b. Cellulose 14. c. Transduction

15. Hot cold phenomenon is seen due to which toxin of staphylococci: [UP 04]

a. Alpha lysin b. Beta lysin
c. Gamma lysin d. Theta lysin

[Ref. Ananthanarayan 8/e, p198, 9/e, p202]

Hot cold phenomenon: Hemolysis initiated at 37°C, but become evident only after chilling

16. Staphalococcus differ from streptococcus by: [Jharkhand 05]

a. Coagulase test b. Catalase test
c. Phosphatase d. Gram negative

[Ref. Harrison 18/e, p1160]

17. Staphylococcus does not produce: [Jimper 04]

a. Hyaluronidase b. Sphingomylinase
c. Hemolysin d. Levococidine

[Ref. Ananthanarayan 8/e, p198, 9/e, p202]

18. Toxic shock syndrome is due to: [Comed 07]

a. Septic abortion b. Forgotten tampons
c. IUCD d. Pelvic examination

[Ref. Ananthanarayan 8/e, p199, 7/e, p182]

19. Most common mechanism of transfer resistance in Staphylococcus aureus is: [DPGEE 08]

a. Conjugation b. Transduction
c. Transformation d. Mutation

[Ref. Jawetz 23/e, p228]

20. Pathogenicity of staphylococci is because of: [PGI 98]

a. Lecithinase
b. M-protein
c. Coagulase
d. Hyaluronidase

[Ref. Ananthanarayan 8/e, p198; 9/e p 201]

21. True statement regarding non-coagulase staphylococci is: [AI 99]

a. They are non-pathogenic
b. They commonly infect indwelling prosthesis
c. They may cause scarlet fever
d. They are separated by gram's staining

[Ref. Ananthanarayan 8/e, p202, 9/e p 206]

22. Common source of staph in hospital: [PGI 99]

a. IV fluids
b. Infective wounds
c. Hands of hospital personnel
d. Bed linen
e. Instruments *[Ref. Harrison 19/e, p955]*

- **MC** mode of spread of S. aureus infection is via hands of health care workers.
- S. aureus is **MC** cause of surgical wound infection and can cross infect wound of other patient too.
- **Most effective** mode of prevention of nosocomial S. aureus infection is **handwashing.**

Answers **15.** b. Beta lysin **16.** b. Catalase test **17.** None **18.** b. Forgotten
19. b. Transduction **20.** c, d **21.** b. They **22.** b. and c

NEET Pattern Questions

1. **Catalase positive, beta – hemolytic – staphylococcus:**
   a. S aureus b. S epidermidis
   c. S saprohyticus d. None
   [Ref. Harrison, 18/e, p1160]

On blood agar S aureus form golden β hemolytic colonies whereas CONS forms small white non-hemolytic colonies

2. **Catalase positive coagulase negative β-haemolytic bacteria:**
   a. Strep pyogens
   b. Staph aureus
   c. Coagulase negative staph
   d. Enterococci *[Ref. Ananthanarayan, 8/e, p202, 9/e, p206]*

3. **A cook is habitual of nose picking while cooking. His clients are at risk for food poisoning with:**
   a. Clostridia difficle b. Staph aureus
   c. Vibrio cholerae d. Bacillus cereus
   [Ref. Ananthanarayan, 9/e, p199]

4. **Staphylococcal scalded skin syndrome is caused by:**
   a. Hemolysin b. Coagulase
   c. Enterotoxin d. Epidermolytic toxin
   [Ref. Ananthanarayan, 9/e, p203]

5. **Blood culture is positive in which infection of staph. aureus:** *[Ref. Ananthanarayan, 9/e, p203]*
   a. TSS b. SSSS
   c. Infective endocarditis d. Impetigo

Blood culture is positive in endarascular infection of bacteremia septicemia, pyema and endocarditis.

6. **All cause Fournier gangrene except:**
   a. Staphylococcus b. Streptococcus
   c. Clostridium d. Bacteroides

Causative organisms of Fournier gangrene: S.aureus, S.pyognes (B hemolytic slreptococci), enterobacteriacae (E.coli Klebsiella, proters), enterococci, pseudomonas and anaerobes like bacteroides and peptostreptococcus

7. **Food poisoning case with diarrhea within 6 hours:**
   a. Staph aureus b. Cl. perfringens
   c. Cl. botulinum d. V. cholerae

8. **For phage typing, how many phages of staphylococcus aureus are used:**
   [Ref. Testbook of Microbiology Chakarborty, 2/e, p242
   a. 12 b. 15
   c. 20 d. 28

A set of over 28 bacteriophage are employed for typing of S.aureus. Most strain are lysed by more than one phage. In India most preventant phage type is 52/52A/80/81.

9. **Protein A of staphylococcus binds to:**
   a. IgA b. IgG
   c. IgD d. IgE

10. **Most common nosocomial infection is:**
    a. Staph aureus b. E. coli
    c. Legionella d. Strep pneumonia
    [Ref. Harrison, 19/e, p955]

11. **Coagulase test differentiates:**
    a. Staphylococci from streptococci
    b. Streptococci from enterococci
    c. Staph aureus from Staph epidermids
    d. Staph epidermidis from staph saprophyticus
    [Ref. Ananthanarayan, 9/e, p200]

12. **Staphylococcus aureus does not cause which of the following skin infection:**
    a. Ecthyma gangrenosum b. Bullous impetigo
    c. Botryomycosis d. Cellulitis

Note: Though ecthyma gangrenosum is classically caused by pseudomonas and gram-negative rods. It can be due to methicillin resistant S. aureus.
Botryomycosis (Bacterial pseudomycosis) rare granulomatous bacterial infection that affects the skin and sometimes viscera. Staph aureus and Pseudomonas aeroginosa are the commonest associated bacteria.

13. **Best available option to prevent MRSA infection in a hospital:**
    a. Prophylactic antibiotics
    b. Fumigation of wards
    c. Proper hand washing
    d. Use of disinfectants *[Ref. Harrison, 19/e, p958]*

14. **Most common site for staphylococcus carrier:**
    a. Skin b. Nose
    c. Oropharynx d. Perineum
    [Ref. Harrison, 19/e, p954]

15. **Test to differentiate staphylococci from micrococci:**
    a. Catalase test
    b. Coagulase test
    c. Novobiosin sensitivity
    d. Oxidation fermentation *[Ref. Ananthanarayan, 9/e, p206]*

16. **Most common cause of artificial heart valve infection: in first 3 months**
    a. Staphylococcus aureus
    b. Streptococcus mutans
    c. Staph epidermidis
    d. Pneumococcus

In initial 12 months MC cause is CoNS, after 12 months etiology is same as native valive endocarditis.

Answers 1. **a.** S aureus 2. **c.** Coagulase negative staph 3. **b.** Staph aureus 4. **d.** Eoidermolytic toxin
5. **c.** Infective endocarditis 6. **c.** Clostridium 7. **a.** Staph aureus 8. **d.** 28 9. **b.** IgG
10. **a.** Staph aureus 11. **c.** Staph aureus from staph epidermids 12. **a.** Ecthyma gangrenosum
13. **c.** Proper hand washing 14. **b.** Nose 15. **d.** Oxidation fermentation 16. **c.** Staph epidermidis

CHAPTER

8

Streptococci

IMPORTANT STREPTOCOCCI AND THEIR CHARACTERISTICS

Species or common name	Lancefield group	Hemolysis	Laboratory test	Common diseases caused
S. pyogenes	A	beta	**Bacitracin sensitive;** PYR test positive; Ribose not fermented	Upper respiratory tract infections; pyoderma; rheumatic fever; glomerulonephritis
S. agalactiae	B	beta	**CAMP test,** hippurate hydrolysis	Neonatal meningitis, septicemia
S. equisimilis	C	beta	Ribose and trehalose fermentation	Pharyngitis, endocarditis
S. anginosus	A, C, F, G, untypable	beta (alpha, gamma)	Group A strains bacitracin resistant, PYR negative	Pyogenic infections
Enterococcus sp. *(E. faecalis and E. faecium E. durans)*	D	gamma (alpha, beta)	**Growth in 6.5% NaCl; PYR positive**	Urinary tract infections, endocarditis, suppurative infections
Nonenterococcal Group D species (S. bovis, S. equinus)	D	gamma	**No growth in 6.5% NaCl**	Endocarditis
Viridans streptococci	Not typed	alpha (gamma)	**Optochin resistant**	Endocarditis *(Str. sanguis);* dental caries *(Str. mutans)*

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Hemolysis:

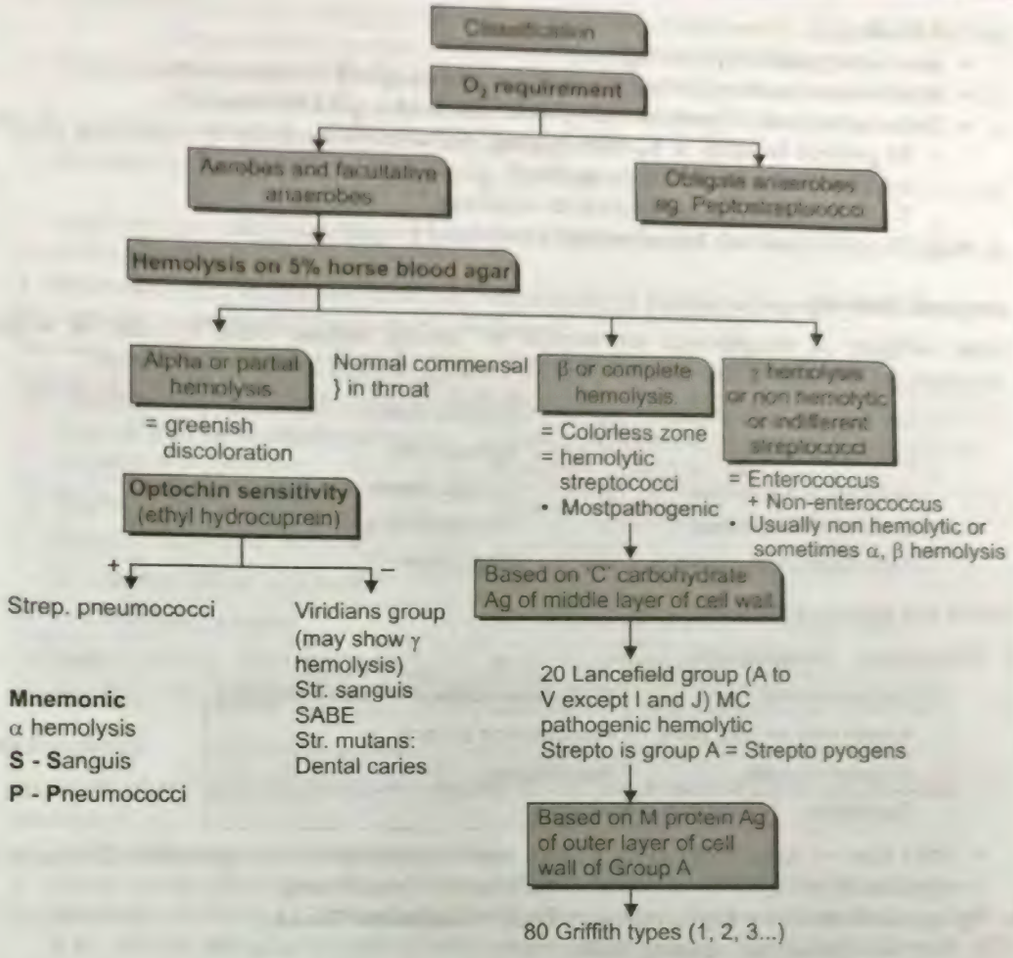
α : S.viridans
β : S. pyogenes
S. agalactiae
S. equisimilis
γ Enterococci

Mnemonic: Careless Lancefield And Manic Griffith
c-carbohydrate → **L**ancefield, **G**roup **A** is classified by **G**riffith on the basis of **M.** Protein.

Pyogenic (Pus generating) Streptococci:
S. Pyogenes
S. agalactiae
S. equisimilis
Mitis (commensal) Streptococci:
S. pneumoniae
S. mitis
S. oralis
S. sanguinis

Mnemonic
α. hemolysis
S. Sanguis
P. Pneumococci

Mnemonic: Careless Lancefield and **M**anic **G**riffith
C – Carbohydrate → Lance field, whereas
M – Protein is the basis of Grif fith classification



Mnemonic
α hemolysis
S - Sanguis
P - Pneumococci

STREP PYOGENES = LANCEFIELD GROUP A

- Can form chain as divide in one plane (*S. aureus* form grape-like cluster as it divide into three planes).
- ***Longest chain*** is produced by non pathogenic *Str. salivarius*.

Cultural Characteristic

- Growth occur only in media containing fermentable carbohydrate or enriched with blood or serum. Growth and hemolysis are promoted by 10% CO_2.

• Virulent strain = Matt finely granular colonies
• Avirulent strain = **Glossy** colonies
• Capsulated (virulent) = **Mucoid** colonies

- Crystal violet agar is a selective medium.

Antigenic Structure

a. ***Polysaccharide capsule:***
- Group A streptococci (GAS) elaborates varying degree of polysaccharide capsule composed of hyaluronic acid. Capsular polysaccharaide plays an important role in protecting GAS from ingestion and killing by phagocytosis. Capsular polysaccharide also plays a role in GAS colonization in the pharynx by binding to CD-44 (a hyaluronic acid binding protein) expressed on human pharyngeal epithelial cell.
- However, hyaluronic acid capsule is a weak immunogen and antibodies to hyaluronate are not protective.

b. *Cell Wall:*
- Inner layer made of peptidoglycan.
- Middle layer made of carbohydrate (basis *of Lancefield classification*).
- Outer layer made of protein and lipoteochoic acid. e.g. M Protein, T, R.
  - **M protein is basis** of **Griffith typing**. *Act as virulence factor* by inhibiting phagocytosis. Antibody to M is protective.
  - T and R protein has no relation to virulence.

c. ***Hair-like pilli (fimbria):*** Important for attachment to epithelial cells.

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> Str. Pyogenes is one of the most prevalent human pathogen

Antigenic Similarity

Some antigens of streptococci are similar to normal human cells, because of which streptococcal infection is associated with autoimmune disease like rheumatic fever.

Capsular hyaluronic acid	→	Synovial fluid
Cell wall protein	→	Myocardium
Group A carbohydrates	→	Cardiac valves
Cytoplasmic membrane	→	Vascular intima
Peptidoglycan (mucoprotein)	→	Skin antigen

Toxins and Virulence Factors

a. **Hemolysin - Streptolysin:**

O [Oxygen labile]	S [Oxygen stable and serum soluble]
• Activity only on pour plate	Hemolysis on surface
• Antigenic specific	Non antigenic
• Cardiotoxic	

- ASO titre → Anti Streptolysin O titre used in retrospective diagnosis; > 200 units is significant; inhibited by cholesterol (but not by Normal sera).

b. **Pyrogenic Exotoxin = Erythrogenic = Dick = Scarlatinal Toxin:**
- This is superantigen causing **TSS.**
- *Dick test* used to identify children susceptible to scarlet fever by intradermal injection. *Schultz Charlton Reaction* is another historic test for diagnosis of scarlet fever.
- **Three types :** Types A ***(MC)*** and Type C are coded by bacteriophage while type B is chromosomal.

c. **Streptokinase (Fibrinolysin)**: Facilitates spread of infection.

d. **Spy Lep:** A serine protease that cleaves and inactivate IL-8, thereby inhibiting neutrophil recruitment to the site of infections.

e. **Deoxyribo nuclease (Streptodornase)**: Responsible for thin serous character of strep exudates. Also called as DNAase.

f. **Nicotinamide Adenine Dinucleotidase** ***(NAD-ase).***

g. **Hyaluronidase:** Favor spread of infection.

h. **Serum opacity factor:** Lipoproteinase.

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> Interaction with host fibronectin (a matrix protein on eukaryotic cells) is considered as the principal mechanism by which Str. Pyogenes binds to epithelial cells of pharynx and skin.

Clinical Manifestations

1. *Respiratory:*
   - ***Sore throat is MC*** streptococcal infection.
2. *Scarlet fever:*
   - Streptococcal pharyngitis, accompanied by rash made up of minute papules giving a characteristic *"Sand paper"* feel to skin.
   - Associated with circumoral pallor, strawberry tongue.
   - Rash reflect hypersenstivity reaction to toxin.
3. ***Skin and Soft tissue infection:***
   - ***Impetigo***: Superficial infection of skin mainly by group A streptococci. Face and leg are *MC site.*
     - Bullous impetigo is caused by Staph. aureus.

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Catalase negative Gr(+) ve cocci from pus is likely to be streptococci.
Bacitracin sensitivity test is the specific test for detection of Group A streptococci (i.e. Str. pyogenes)

- ***Cellulitis:*** Diffuse spreading infection of skin and subcutaneous tissue especially of leg. Caused by *Str. pyogenes* (***MC***), *Staph, Cl. perfringens, E.coli.*
- ***Erysipelas***: Superficial form of cellulitis with bright red appearance of involved skin, seen classically on cheek. Skin assumes peau-d-orange texture due to involvement of superficial lymphatics.
- ***Necrotizing fascitis***: Caused by Group A streptococci called as *Hemolytic streptococcal gangrene.*
- *In skin infection ASO titre is not high and so ASO estimation has not much clinical significance.*

4. ***Genital infection:***
   - **Anaerobic streptococci** are most important cause of **puerperal sepsis.**
5. **Bacteremia, pneumonia, toxic shock syndrome.**
6. ***Non suppurative complication:*** Develop 1-3 weeks after acute infection.

Acute rheumatic fever	Acute glomerulonephritis
• Develop after throat infection by any serotype of *S. pyogenes*	• Develop after either sore throat or skin infection by serotype 49, 53-55, 59-64, 1 and 12
• Repeated attacks common	• No repeated attacks
• Penicillin prophylaxis indicated	• Not indicated
• Course is progressive or static	• Spontaneous resolution
• ASO titre always raised	• May or May not (after skin infection) raised
• Marked immune response with no change in complement level	• Moderate immune response with decrease in complement level.

Lab Diagnosis

- In acute pharyngitis diagnosis is established by throat swab culture (diagnostics gold standard).
  - *Pike's medium* is used as *transport media.*
  - Sheep blood agar is recommended for isolation as it is inhibitory for *H. hemolyticus.*
- In Rheumatic fever and Glomerulonephritis retrospective diagnosis is needed.
  - ***ASO titre*** > 200 is indicative of prior streptococcal disease. After skin infection ASO titre is usually low. So useful only in retrospective diagnosis of rheumatic fever.
  - Organism are not detectable in acute rheumatic fever or acute glomerulonephritis. These lesions are believed to be due to hypersensitivity to some streptococcal component with antigenic cross reaction.
  - *In acute glomerulonephritis and pyoderma, Anti-DNAse and antihyaluronidase are used for retrospective diagnosis.*
- Streptozyme test: *Passive slide haemagglutination* test. Sensitive and specific test for all types of streptococcal infection.

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CAMP reaction is the specific test for detection of Group B streptococci.

Treatment of Group A Streptococci	
• *Pharyngitis, impetigo, erysipelas,* cellulitis	– **Penicillin (Pn)**
• *Necrotizing fascitis / myositis*	– **Surgical debridement + Pn + Clindamycin**
• *Pneumonia / Empyema*	– **Pn + empyema drainage**
• *Streptococcal TSS*	– **Penicillin + Clindamycin + iv Ig**

OTHER HEMOLYTIC STREPTOCOCCI (= LANCEFIELD B HEMOLYTIC STREPTOCOCCI)

Group B (Strep. agalactiae)

- *S. agalactiae* is the ***MC*** cause of neonatal meningitis in west.
- It **does not** hydrolyse bile esculin agar, however hydrolyse sodium hippurate and are bacitracin resistant.
- *Virulence factor* is ***polysaccharide capsule.*** *(Group A have hyaluronic acid capsule).*
- Identified by positive **CAMP** *(Christie, Atkins and Munch-Peterson)* **reaction (CAMP factor is a phospholipase).** *... Harrison 18/e, p 1178*

- In infant it cause 2 type of infection:
  - ***Early Onset:*** More common, acquired from maternal vagina during birth.
  - Essentially all infants are bacteremic presenting with lethargy, respiratory distress and hypotension.
  - ***Late Onset Infection:*** Infection more often obtained from environment.
  - Meningitis is *the MC* manifestation and in most cases is associated with it strain of *capsular type III.*
- ***Treatment***
  Penicillin is *DOC* for all group B streptococcal infection.

GROUP C -STREP. EQUISIMILIS

- Predominant animal pathogen and is the source of streptokinase used for thrombolytic therapy.

GROUP D - STREPTOCOCCI (NON HEMOLYTIC)

Includes:

- Fecal streptococci/*Enterococcus.*
- Non enterococcal group D streptococci.
- Enterococci are now considered as separate genus on the basis of DNA homologus studies. *... Harrison 19/e, p 971*

Distinctive Features of *Enterococcus:*

- Grow in 40% bile and hydrolyse esculin *... Jawetz 27/e, p 226*
- Grow in 6.5% NaCl at pH 9.6, 45°C and in 0.1% methylene blue milk
- Majority of clinically significant enterococci hydrolyze PVR. This is helpful in differentiating them from S. bovis.
- Heat resistant, surviving 60°C for 30 minutes
- Non-hemolytic to ovine or bovine RBC (commonly used in lab). However some strains of E. faecalis can lyse human RBC *... Harrison 19/e, p 971*
- Majority of enterococcal species hydrolyze PYR, this feature distinguish them from organism of Str. bovis group.
- Present in intestine, genital tract and saliva
- Cause wound infection, UTI, nosocomal bacteremia in patient with IV catheters, endocarditis.
- Usually resistant to Penicillin, cephalosporin, etc
- In case of Vancomycin and β lactam resistance - Linezolid (against all enterococci) or quinupristin, dalfopristin (against E. faecium only) are given.

Non Enterococcal Group D Streptococci

- Grow in bile and hydrolyze esculin (bile-esculin positive) *... Jawetz 27/e, p 220*
- Unable to grow in 6.5% NaCl and PYR negative (*difference from enterococci*)
- Penicillin susceptible.
- Main species causing infection is *S.bovis. S.bovis* endocarditis is often associated with neoplasm of GIT, most commonly colon.

GROUP F - STREPTOCOCCI

- Called as minute streptococci.
- Includes *Streptococcus* MG.
- Isolated from cases of atypical pneumonia.

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- Enterococci are identified by their ability to grow on bile contaning medium.
- E. faecalis and E. faecium are the important enterococci causing human disease.
- Vancomycin resistance is seen in:
  - 80% of E. faecium
  - 7% of E. faecalis

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Virulence factors of enterococci

a. Enterococal secreted factors:
   - Enterococcal hemolysin
   - Enterococcal protease (GelE and SprE)

b Enterococcal surface components:
   - Surface pili
   - Surface protein

c. E. faecalis stress protein

VIRIDANS STREPTOCOCCI

- Heterogenous group of organism that are the commonest agent causing bacterial endocarditis. These are part of normal flora of mouth.
- Species include:
  - *S.sanguis:* MC viridans streptococcus associated with endocarditis.
  - *S.mutans:* Important cause of dental caries.
  - *S.salivarus.*

PNEUMOCOCCUS

- Gram positive Lanceolate diplococci. *Possess polysaccharide capsule.*
- It is *MC* cause of *Lobar pneumonia, sinusitis, otitis media.*
- It is *MC* cause of *bacterial meningitis* in adults.

Fermentation ability of pneumococci is tested in Hiss's serum sugars

Characteristic	Pneumococci	Streptococci
Morphology	Capsulated	Mostly non capsulated
Quellung test	+ ve	– ve
Colonies	Draughtsman colonies	Dome-shaped colonies
Bile solubility	++	–
Inulin fermentation	++	–
Optochin sensitivity	++	–
Intraperitoneal Inoculation of mice	Fatal infection	Not pathogenic
Growth in liquid media	Uniform turbidity	Granular turbidity or powdery deposit.

Morphology and Culture

- Capsule enclose a pair of flame shaped Lanceolate bacteria.
- Grow only in enriched media.
- Fastidios in nature. Grows best in 5% CO_2 ... *Harrison 19/e, p 946*
- On blood agar they are α hemolytic and on prolong incubation colonies show *draughtsman or carrom coin appearance.* Under anaerobic conditions produce β hemolysis.
- Strains with ***abundant capsular material*** *(3, 7)* form large, mucoid colonies.
- Pneumococci readily undergo autolysis in culture due to presence of autolytic amidase which is activated by bile salts; hence *they are bile soluble.*
- *Bile solubility* is of *diagnostic importance.*
- *Inulin fermenter (useful test for differentiating from streptococci as they are unable to do so).*

Virulence factors

- Capsular polysaccharide = specific soluble substance:
  - *MC* antigen and virulent factor.
  - Protects against phagocytosis.
  - *Type 3* pneumococci has abundant capsular material, *so more virulent.*
- *C substance ("cell wall" substance):*
  - Unique to penumococci. It is a polysaccharide consisting of teichoic acid with a phosphoryl choline residue. These choline residue provide attachment to potential virulence factors such as pneumococcal surface protein A (PspA) and pneumococcal surface adhesin A (psaA).
- *Penumolysin:*
  - Membrane damaging toxin which has cytotoxic and complement activating properties.
- *Autolysin.*
- *IgA_1 Protease:*
  - Pneumococci produce an extracellular protease, that specifically cleaves human IgA_1. IgA1 protease enable pneumococci to establish infection in upper respiratory tract, where IgA predominates.

Quellung reaction

On mixing pneumococci with specific or polyvalent antipolysaccharide serum the capsule becomes apparently swollen, sharply delineated and refractile. This reaction is used for ***rapid identification.***

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Quellung reaction:
Rapid identification of pneumococci

Risk factor for pneumococcal infection

- ***Respiratory infection Inflammation***
  - Influenza, other viral respiratory infection
  - Allergies
  - Cigarette smoking
  - Chronic obstructive pulmonary disease
- ***Anatomical disruption of meninges (dural tear)***
- ***HIV infection***
- ***Defective antibody formation***
  - Selective IgG subclass deficiency
  - Multiple myeloma
  - Chronic lymphocytic leukemia
  - Lymphoma
- ***Defective complement function***
- ***Defective clearance of pneumococcal bacteremia***
  - Congenital asplenia, hyposplenia
  - Splenectomy
  - Sickle cell disease
- ***Other conditions***
  - Alcoholism
  - Malnutrition
  - Glucocorticoid treatment
  - Cirrhosis of the liver
  - Renal insufficiency
  - Diabetes mellitus
  - Anemia
  - Coronary artery disease

Manifestations

- *Commonest* pneumococcal infection are **otitis media** and **sinusitis.**
- *Meningitis* is ***most serious*** pneumococcal infection. Pneumococci is the commonest cause of meningitis in alcoholics.
- Pneumonia: Mostly due to types 1 to type 8 strains.
  - *Type 3 strain is **most virulent.***
  - ***MC*** complication of pneumococcal pneumonia—***Empyema***
- *S. pneumoniae* are ***MC*** cause of sepsis in splenectomized patient.
- ***Austrain syndrome:*** Concurrence of pneumococcal pneumonia, endocarditis and meningitis.

Diagnosis

- Gram's staining and culture of sputum or CSF.
- *Gold standard* for diagnosis of pneumococcal pneumonia is pathologic examination of lung tissue.
- **Biomarkers** - **CRP:** testing by passive agglutination
  - Raised procalcitonin level is another biomarker which gets elevated in invasive pneumococcal disease.

Treatment

- *Otitis media/Sinusitis/ Pneumonia*—**Amoxicillin.**
- *Meningitis*—**Ceftriaxone + vancomycin.**
- *Endocarditis*—**Ceftriaxone or cefotaxime + vancomycin.**
- *Penicillin resistance is due to alteration in penicillin binding protein (not due to production of β lactmase).*

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Commonest pneumococcal infection:
- Otiis media\
- Sinusitis

Most serious pneumococcal infection: Meningitis
Most virulent pneumococcal strain type 3 strain

Pneumococcal Vaccine

Two types are available:

a. Polyvalent polysaccharide vaccine:
   - Contains capsular antigen of ***23 most prevalent serotypes.***
   - ***Protection rate—80 - 90%***

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Pneumococcal vaccine:
- Polyvalent polysaccharide vaccine
- Polysaccharide protein conjugate vaccine

PNEUMOCOCCAL VACCINE	
Indication	**Contraindication**
• In pateint with absent or dysfunctional spleen	• < 2yr child
• Sickle cell disease	• Lymphoreticular malignancies and immunosuppressive therapy
• Coeliac disease	• CSF leak
• Chronic renal, lung, heart and liver disease	• Alcoholic cirrhosis
• Diabetes mellitus	• Multiple myeloma
• Immunodeficiencies including HIV	• Chronic glucocorticoid therapy
• Routinely as ≥ 65 years of age	• 'Hodgkin's'disease
	• Organ transplant recepient

b. Polysaccharide protein conjugate vaccine.
- As infants and young children responds poorly to polysaccharide vaccine, protein conjugate vaccines were developed.
- Till 2010, three PCV products containing 7, 10 and 13 serotypes were commercially available.
- PCV are recommended by WHO for inclusion in routine childhood immunization schedule worldwide.

Splenectomy
- Splenectomy increase the risk of following infections: streptococcus pneumococcal, Hemophilus influenzae a some Gram negative enteric organisms.
- Splenectomized patient should be vaccinated against Pneumococi Haemophilus influenzae, Meningococci
- In splenectomized individual any unexplained fever is medical emergency.

Multiple Choice Questions

GENERAL

1. **Which is false regarding gram-positive cocci?**
   a. *Staph. saprophyticus* causes UTI in females
   b. Micrococci are oxidase positive **[AI 08]**
   c. Most enterococci are sensitive to penicillin
   d. Pneumococci are capsulated

2. **A patient of RHD developed infective endocarditis after dental extraction. Most likely organism causing this is: [AIIMS 01]**
   a. *Streptococcus viridans*
   b. *Streptococcus pneumoneae*
   c. *Streptococcus pyogenes*
   d. *S. aureus*

3. **Streptococci causing dental caries: [PGI 96, 01]**
   a. Streptococci equisimilis
   b. Streptococci mutans
   c. Streptococci pneumoniae
   d. Streptococci bovis

4. **In a case of neonatal meningitis, the etiologic bacteria was found to have properties of β-hemolysis, bacitracin resistance, CAMP positive. Which of the following is most likely causative agent? [AI 2010]**
   a. S. pyogenes b. S. agalactiae
   c. S. pneumoniae d. E. faecalis

5. **Eight months after prosthetic valve replacement, most common organism causing infective endocarditis is: [AIIMS Nov. 10]**
   a. Staph. epidermidis b. Strep. viridans
   c. Staph. aureus d. HACEK

STREPTOCOCCI PYOGENES

6. **Which component of *St. pyogenes* has cross reactivity with synovium of human ? [AI 08]**
   a. Capsular hyaluronic acid
   b. Cell proteins
   c. Group A carbohydrate antigens
   d. Peptidoglycan

7. **A boy with skin ulcer on leg, culture revealed beta hemolysis. School physician said that similar hemolysis was seen in organism from sorethroat, what is the similarity between both? [AIIMS Nov. 10]**
   a. A protein is same for both
   b. C carbohydrate antigen is different
   c. C carbohydrate antigen is the same
   d. Strain causing both are same

8. **A person from village is complaining of development of pustules. Extract from pus has shown gram positive cocci, showing hemolysis, catalase –ve, identified as a group of streptococci. Following test is used:**
   a. Bacitracin sensitivity **[AI 07; AIIMS Nov. 06]**
   b. Novobiocin sensitivity
   c. Optochin sensitivity
   d. Hemolysis

9. **All are true about *Streptococcus* except: [AI 01]**
   a. M-protein is responsible for production of mucoid colonies
   b. M- protein is responsible for virulence
   c. Mucoid colonies are virulent
   d. No resistance to penicillin has been reported

10. **Toxin involved in the streptococcal toxic shock syndrome is: [AI 01]**
    a. Pyrogenic toxin b. Streptokinase
    c. Hemolysin d. Neurotoxin

11. **True statement about antistreptolysin 'O' titre is: [AIIMS, 011, AI 97]**
    a. In normal people the titre is < 200
    b. In acute glomerulonephritis the titre is low
    c. ASO titre >200 indicate rheumatic fever
    d. Streptozyme test is an active hemagglutination test

12. **Lance field grouping of streptococci is done by using: [AIIMS 07]**
    a. M protein
    b. Group C peptidoglycan cell wall
    c. Group C carbohydrate antigen
    d. M antigen

13. **An outbreak of Streptococcal pharyngitis has occured in a remote village. In order to carry out the epidemiological investigations it is necessory to perform the culture of the throat swab of the patient suffering from the disease. The transport media of choice would be: [AIIMS 02]**
    a. Salt manitol media
    b. Pike's media
    c. Stuart media
    d. Carry Blair media

14. **Streptococcal toxic shock syndrome is due to the following virulence factor: [AIIMS 03]**
    a. M-protein b. Pyrogenic exotoxin
    c. Streptolysin O d. Carbohydrate cell wall

15. **The commonest organism causing cellulitis is: [AIIMS 02]**
    a. *Streptococcus pyogenes*
    b. *Streptococcus faecalis*
    c. *Streptococcus viridans*
    d. Microaerophilic streptococci

16. **True about streptococcus:** [PGI 03]
    a. Lancefield classification is based on M protein
    b. Group 'G' not found in human
    c. Group 'B' causes neonatal meningitis
    d. Group 'C' can be isolated from vaginal flora
    e. Group 'D' causes urinary tract infection

17. **Streptococcus all are true except:** [AIIMS May 10, May 11]
    a. Streptodornase cleaves DNA
    b. Streptolysin O is active in reduced state
    c. Streptokinase is produced from serotype A, C, K
    d. Pyrogenic toxin A is plasmid mediated

18. **Antigenically similar to Streptolysin O:**
    a. Clostridial perfringenes toxin [PGI 2010, 2009]
    b. Tetanolysin
    c. Botulinum toxin
    d. Erythrotoxin

19. **A 5-year-old child presents with pustular lesions on the lower legs. The culture from the lesion showed hemolytic colonies on blood agar which were Gram-positive cocci. Provisional diagnosis of Group A streptococcal pyoderma can be done by?** [AI 2012]
    a. Catalase positivity b. Optochin sensitivity
    c. Bile solubility d. Bacitracin sensitivity

20. **Group A streptococci is best siagnosed by:**
    a. Optochin sensitivity [AIIMS Nov 2011]
    b. Bacitracin Sensitivity
    c. Catalase negative
    d. Bite solubility

ENTEROCOCCI

21. **Which of the following is not true regarding enterococcus ?** [AI 08]
    a. Common species are *E. faecalis* and *E. faecium*
    b. It is a cause for peritonitis
    c. It is universally susceptible to penicillins
    d. Can cause intra-abdominal abscess

22. **A beta hemolytic bacteria is resistant to vancomycin, shows growth in 6.5% NaCl, is non bile sensitive. It is likely to be:** [AI 01]
    a. *Strep. agalactiae* b. *Strep. pneumonia*
    c. *Enterococcus* d. *Strep. Bovis*

23. **A patient admitted to an ICU is on central venous line for the last one week. He is on ceftazidime and amikacin. After 7 days of antibiotics he develops a spike of fever and his blood culture is positive for gram positive cocci in chains which are catalase - negative. Following this vancomycin was started but the culture remained positive for same organism even after 2 weeks of therapy. The most likely organism causing infection is:** [AI 07; AIIMS 06]
    a. *Staphylococci aureus*
    b. *Viridans streptococci*
    c. *Enterococcus faecalis*
    d. Coagulase negative *Staphylococcus*

24. **Which of the following organism, when isolated in the blood, requires the synergistic activity of penicillin + amino glycoside for appropriate therapy:** [AIIMS 04]
    a. *Enterococcus faecalis*
    b. *Staph. aureus*
    c. *Streptococcus pneumoniae*
    d. Bacterioides fragilis

25. **45 years old Ramlal has intra abdominal sepsis. The causative organism was found to be vancomycin, gentamycin and ampicillin resistant. It grows well in presence of 6.5% NaCl and arginine. Bile esculin hydrolysis is positive. Which of the following is this organism ?**
    a. *Strept. agalactiae* [AIIMS 01]
    b. *Enterococcus fecalis*
    c. *Streptococcus bovis*
    d. *Streptococcus pneumoneae*

PNEUMOCOCCI

26. **An infant had high grade fever and respiratory distress at the time of presentation to the emergency room. The sample collected for blood culture was subsequently positive showing growth of α-hemolytic colonies. On Gram staining these were Gram-positive cocci. In the screening test for identification, the suspected pathogen is likely to be susceptible to the following agent:** [AI 07, AIIMS May 2012]
    a. Bacitracin b. Novobiocin
    c. Optochin d. Cloxacillin

27. **In a patient of orbital cellulitis, micro organism on culture show greenish colonies and optochin sensitivity. The most likely organism is:** [AI 00]
    a. *Strep. viridans* b. *Staphylococcus*
    c. *Pseudomonas* d. *Pneumococcus*

28. **True statement regarding Pneumococcus is:** [AI 00]
    a. Virulence is due to polysaccharide capsule
    b. Capsule is protein in nature
    c. Antibodies against capsule are not protective
    d. Resistance to penicillin has not yet been reported

29. **An infant had high grade fever and respiratory distress at the time of presentation to the emer-gency room. The sample collected for blood culture was subsequently positive showing growth of α-hemolytic colonies. On Gram staining these were gram-positive cocci. In the screening test for identification, the suspected pathogen is likely to be susceptible to the following agent:** [AIIMS 07, 06]
    a. Bacitracin b. Novobiocin
    c. Optochin d. Oxacillin

30. **The sputum specimen of a 70 year old male was cultured on a 5% blood agar. The culture showed the**

presence of α-hemolytic colonies next day. The further processing of this organism is most likely to yield: [AIIMS 05]

a. Gram-positive cocci in short chains, catalase negative and bile resistant
b. Gram-positive cocci in pairs, catalase negative and bile soluble
c. Gram-positive cocci in clusters, catalase positive and coagulase positive
d. Gram-negative coccobacilli, catalase positive and oxidase positive

31. Most common causative organism for lobar pneumonia is: [AIIMS 04]

a. *Staphylococcus aureus*
b. *Streptococcus pyogenes*
c. *Streptococcus pneumoniae*
d. *Haemophilus influenzae*

32. After Splenectomy, most common infection:

a. Pneumococcal b. *E. coli* [PGI 00]
c. *Klebsiella* d. *Streptococcus*

33. A 65-year-old man presents with complaints of chest pain, fever, cough with sputum. Sputum examination reveals pus cells with Gram positive cocci. Blood agar showed positive results. How will you differentiate this from other Gram positive cocci. [AIIMS Nov. 2009]

a. Bacitracin sensitivity b. Optochin sensitivity
c. Bile solubility d. Positive coagulase

34. An eight year old child with history of pain and discharge from right ear presents with fever, neck rigidity and a positive Kerning's sign. Discharge was stained with Gram stain which revealed gram positive cocci. Which of the following is the most likely organism.

a. H. influenzae [AI 2011]
b. Staphylococcus
c. Pneumococcus
d. Pseudomonas

35. All are true about streptococcus preumoniae except:

a. Capsule aids in infection [AI 2011]
b. Commonent infection is otitis media
c. Respiratory tract of carriers is most important source of infection
d. Meningitis caused by S. pneumoniae is milder than others

36. A person presents with pneumonia. His sputum was sen for culture. The bacterium obtained was gram positive cocci in chains and alpha haemolytic colonies on sheep agar. Which of the following will help in confirming the diagnosis: [AIIMS May 2012]

a. Novobiocin b. Optochin
c. Bacitracin d. Oxacillin

Explanations and References with Illustrative Answers

1. Ans. (c) Most enterococci are sensitive to penicillin

Ref. Harrison 19/e, p 974, 18/e, p 1187; Ananthanarayan 8/e, p 216, 9/e, p 217

- Unlike streptococci, enterococci are not reliably killed by penicillin or ampicillin alone at concentration achieved clinically in the blood or tissues.
- Antimicrobial susceptibility testing should be performed routinely on enterococcal isolates.

Enterococci resistance to penicillin is via two mechanism:

Penicillin Resistance	
Penicilase production	**Altered penicillin binding protein**
• Seen in *E. faecalis*	• Common in *E. faecum*
• Vancomycin, ampicillin/sulbactam, amoxicillin/clavulanate, imipenem may be used in combination with gentamycin	• Vancomycin plus gentamycin is used

Other options

Option 'a'

- *Staph. saprophyticus* specially causes UTI in sexually active young women.

Option 'b'

- Micrococci are catalase and oxidase positive Gr +ve cocci. They are strict aerobes and are non-pathogenic.
- Micrococci are differentiated from Staphylococci by Hugh and Deifson oxidation fermentation test in which micrococci shows oxidative pattern while staphylococci show fermentative pattern.

Anaerobic Cocci		
Features	**Gram +ve**	**Gram –ve**
Organism	• ***Peptostreptococci***	***Veillonellae***
	• ***Peptococcus***	
Inhabitation	• Intestine, vagina and mouth	Mouth, intestine and genital tract
Diseases	• ***Puerperal sepsis***	No disease is identified till now.
	• Visceral abscess	
	• UTI, wound infection	
	• Gangrenous apendicitis	
Treatment	• Sensitive to penicillin, chloramphenicol and metronidazole	

2. Ans. (a) *Streptococcus viridans* *Ref. Harrison 17/e, p 789, 18/e, p 1053, 1171; Jawetz 27/e, p 782; CMDT 2014, p 1388*

This is case of ***subacute bacterial endocarditis (SABE)*** *as patient has rheumatic heart disease (so damaged heart valves).*

Endocarditis					
	Native valve		**Prosthetic valve**		**Injection drug users**
	Community associated	*Nosocomial*	*Early (<12 Month)*	*Late (>12 month)*	
Most common organism	Staph.aureus	Staph aureus	Coagulase (–)ve staph	Viridans Streptococci	*Staph. aureus* (usually right sided)

Remember
- Staph. aureus is now the leading cause of native valve endocarditis — CMDT 2014, p 368
- ***MC*** cause of SABE – S. viridans
- ***MC*** cause of acute bacterial endocarditis – S. aureus
- ***MC*** cause of prosthetic valve endocarditis – S. epidermidis
- ***MC*** cause of endocarditis in iv drug users – S. aureus

3. **Ans. (b)** ***Streptococci mutans*** *Ref. Ananthanarayan 8/e, p 206, 9/e, p 218*

S. mutans:

- It is member of viridans streptococci (α-hemolytic) which is part of the normal flora of the oral cavity.
- It assumes bacillary form in acid environment.
- Can synthesize acid and large polysaccharide (adhesive dextran or levan) from sucrose.

4. **Ans. (b) S. agalactiae** *Ref. Ananthanarayan 8/e, p 212-213, 9/e, p 216*

- *S. agalactiae* is the ***MC*** cause of neonatal meningitis in west.
- It does not hydrolyse bile esculin agar, however hydrolyse sodium hippurate and are bacitracin resistant.
- Identified by **CAMP** *(Christie, Atkins and Munch - Peterson)* **reaction.**
- In infant it cause 2 type of infection :
  - a. ***Early Onset*** : – More common, acquired from maternal vagina during birth.
    – Essentially all infants are bacteremic presenting with lethargy, respiratory distress and hypotension.
  - b. ***Late Onset Infection*** : – Infection more often obtained from environment.
    – Meningitis is *the* ***MC*** manifestation and most cases are associated with strain *capsular type III.*
- Other Group B infections in neonates include arthritis, osteomyelitis, conjunctivitis, peritonitis, omphalitis, and endocarditis.
- Adult infection include puerperal sepsis and pneumonia.
- ***Treatment:*** Penicillin is ***DOC*** for all group B streptococcal infection.

5. **Ans. (a) Staph. epidermidis** *Ref. Harrison 19/e p 817; 18/e p 1053*

- Coagulase negative staphylococci is the most common cause of endocarditis in posthetic value recepients with in one year of surgery. *For full explanation refer previous answers.*

6. **Ans. (a) Capsular hyaluronic acid** *Ref. Ananthanarayan 8/e, p 208, 9/e, p 212; Harsh Mohan 5/e, p 330*

Cross reactivity of Streptococcal antigen	
Bacterial antigen	***Cross reacting human component***
Capsular hyaluronic acid	Human synovial fluid
Group A carbohydrate antigen	Cardiac valves
Cytoplasmic membrane antigen	Vascular intima
Cell wall protein	Myocardium
Peptidoglycan	Skin antigen
Membrane antigens	Sarcolemma of smooth and cardiac muscle, dermal fibroblasts and neurons of caudate nucleus

7. **Ans. (c) C-carbohydrate antigen is the same** *Ref. Jawetz 25/e p 195, 196, 27/e p 215*

β hemolytic streptococci causing both skin infection and sore throat can be none other than Group A streptococci.

- The basis by which Lancefield classified streptococci in to groups A-H and K-V is c carbohydrate. So, different Group A streptococci have same c carbohydrate. In group A, c carbohydrate is rhannose – N-acetyl glycosamine.
- Based on the M protein Griffith divided Group A carbohydrate in to 80 subtypes. Sore throat strains and skin strains of streptococci differ in their M-protein. Skin infection is usually caused by M-types 49,57, 59-61.

8. **Ans. (a) Bacitracin sensitivity** *Ref. Ananthanarayan 8/e, p 207, 9/e, p 210*

"Sensitivity to bacitracin is employed as a convenient method for differentiating Str. pyogenes from other hemolytic streptococci."

Pustule – A vesicle filled with leukocyte. MC cause is virus. MC bacterial cause is *Strep. pyogenes.*

Remember:
- *Str. pyogenes is the etiologic agent in most of the streptococcal skin infection.*
- *Vancomycin is the drug of choice.*
- *Optochin sensitivity* and bile solubility is used to differentiate pneumococci from *Strep. viridans* group.

9. **Ans. (a) M-protein is responsible for production of mucoid colonies**
Ref. Ananthanarayan 8/e, p 207, 9/e, p 212; Jawetz p 25/e, p 198, 27/e p 215

"Mucoid colonies are due to production of capsule of hyaluronic acid not due to M-protein." .... *Harrison 19/e, p 964*

Growth characteristic of *Strep. pyogenes:*

- On blood agar *virulent stains* forms **matt or mucoid colonies** while *avirulent* form **glossy colonies.**
- M, T, R are proteins found on outer part of cell wall and forms the basis of **Griffith classification.**
  - **M protein** - Acts as virulence factor and antibody against it are protective.
  - **T and R protein** - No relation to virulence
- Streptococci (except enterococci) are usually susceptible to penicillin so *option "d"* is not completely wrong. As *option "a"* is completely wrong, that would be the answer.

10. Ans. (a) Pyrogenic toxin *Ref. Jawetz 25/e, p 198, 27/e 218*

"Streptococcal TSS is caused by pyrogenic exotoxin."

Pyrogenic exotoxin = Erythrogenic toxin = Dick = Scarlatinal toxin

- Produced by group A streptococci (type 1, 2, 3, 12 and 28)
- Associated with streptococcal toxic shock syndrome and scarlet fever.
- There are 3 type of pyrogenic exotoxin: A, B, and C
- A (*MC* cause of streptococcal TSS) and C are coded by bacteriophage gene, while B is chromosomal
- Acts as superantigen
- Associated with streptococcal toxic shock syndrome and scarlet fever
- *DOC* for streptococcal TSS is – **Clindamycin** ..... *Harrison 19/e, p 969, 18/e, p 1177*

11. Ans. (b) In acute glomerulonephritis the titre is low *Ref. Ananthanarayan 8/e, p 212, 9/e, p 216*

Retrospective diagnosis of streptococcal infection

- ***ASO (Anti Streptolysin 'O') titre:***
  - Estimation of antibody against streptolysin is a standard serological test for retrospective diagnosis of streptococcal infection.
  - ASO titre > 200 are indicative of prior streptococcal infection.
  - Raised after throat infection only
  - ***Acute rheumatic fever:*** – High level of ASO titre are usually found
    – Titres > 300 or 350 are taken as significant.
  - ***Acute glomerulonephritis:*** – ASO titres are often low.
- ***Streptozyme test***
  - Passive slide hemagglutination test
  - Becomes positive after nearly all types of streptococcal infection whether of throat or skin.
- ***Anti DNA ase B and Antihyaluronidase***
  - Useful for retrospective diagnosis of streptococcal pyoderma or for acute glomerulonephritis for which ASO titre is of much less value.

12. Ans. (c) Group C carbohydrate antigen *Ref. Ananthanarayan 8/e, p 205, 9/e, p 209*

Lancefield classification: Classification of β-hemolytic streptococci into Group A to V (except I, J) on the ***basis*** of **group specific C carbohydrate.**

Griffith classification: Serological typing of group A streptococcus pyogenes on the ***basis*** of **M proteins into types 1, 2, 3, etc.**

Group C Carbohydrate

- Present in middle layer of cell wall.
- This antigen is an integral part of cell wall.
- Serological grouping of streptococci depend on C carbohydrate for which it has to be extracted from cell wall.
- ***Method for extraction are:***
  - Lancefield's acid extraction method (organism are grown in Todd Hewitt broth)
  - Fuller's method
  - Maxted's method
  - Rantz and Randall's method (Autoclaving).

Streptococcal Group C Carbohydrate

Group A	Rhamnose N-acetyl glucosamine
Group B	Rhamnose glusamine Polysaccharide
Group C	Rhamnose N-acetylgalactosamine
Group D	Glycerol teichoic acid

13. Ans. (b) Pike's media *Ref. Ananthanarayan 8/e, p 211-212, 9/e, p 215*

"Pike's media is transport media for Streptococci."

Throat culture is the gold standard for diagnosis of streptococcal pharyngitis

Diagnosis of Streptococcal Pharyngitis

- **Specimen:**
  - Throat specimen collected by vigorous rubbing of a sterile swab over both tonsillar pillars
- **Culture**
  - Throat culture is diagnostic gold standard for pharyngitis.
  - Swab are either plated immediately or sent to laboratory in Pike's medium (used as transport media).
  - Specimen is plated on blood agar and incubated at 37°C anaerobically or under 5-10% CO2.
  - Sheep blood agar is recommeded for primary isolation because it is inhibitory for Haemophilus.
- Latex agglutination or enzyme immunoassay of swab specimen is a useful adjunct to throat culture. These rapid diagnostic tests are highly specific but less sensitive. So, a positive result is an indication for treatment, but negative result should be confirmed by throat culture.

Remember:
- Stuart's medium is transport media for Gonococci.
- Cary - Blair medium is transport media for V. cholera.

14. Ans. (b) Pyrogenic exotoxin *Ref. Jawetz 27/e, p 218, 216*

Remember:
- **MC** cause of toxic shock syndrome – Staphylococci
- **MC** cause of streptococcal TSS – Pyrogenic exotoxin A = erythrogenic / Dick / Scarlatinal toxin.
- **MC** cause of staphylococcal TSS – TSST = Pyrogenic exotoxin C = Enterotoxin type F.
- **DOC** of TSS (both streptococcal and staphylococcal) – Clindamycin.

15. Ans. (a) *Streptococcus pyogenes* *Ref. Harrison 19/e, p 967, 18/e, p 1175*

Cellulitis:
- Diffuse spreading infection of skin (dermis and subcutaneous tissue) especially of lower leg.
- Caused by *Strep pyogenes* **(MC)**, Staph, *Cl perfringens, E.coli.*
- Major portal of entry for lower leg cellulitis is toe web *tinea pedis* with fissuring of skin.
- Skin become peud orange in appearance; recurrent attack may sometimes affect lymphatic vessels producing permanent swelling called as *solid edema.*
- Streptococcus cellulitis tends to develop at sites where lymphatic drainage is disrupted.

16. Ans. (c) and (e) Group B cause neonatal meningitis and Group D cause UTI *Ref. Ananthanarayan 8/e, p 206, 9/e, p 210*

Streptococcal	Organism	Disease
Lancefield		
Group A	S. pyogenes	RTI, pyoderma, rheumatic fever, glomerulonephritis
Group B	S. agalactiae	Neonatal meningitis
Group C	Strepto. equisimilis	Pharyngitis, endocarditis
Group D	Enterococcal	UTI, endocarditis
	Non-enterococcal	Endocarditis
Group F	Minute streptococci	Rarely cause atypical pneumonia
Group G	Commensal in throat	Tonsillitis, endocarditis, UTI
Not typed	Viridans Streptococci	Endocariditis (by S.sanguis)
		Dental caries (by S.mutans)

Note:
- Streptococcus commensal in throat: S. pyogenes, S. equisimilis.
- Streptococcus commensal in female genital tract: Str. agalactiae, viridans strep.

17. Ans. (d) Pyrogenic toxin A is plasmid mediated *Ref. Ananthanarayan 8/e, p 208, 9/e, p 213*

Let us cnsider each option

Option 'a'

STREPTODORNASE Enzyme that Degrades DNA			
Four types (ABCD) **Type B is most antigenic**	• Liquifies thick pus • Responsible for serous character of streptococcal pus	• Anti DNAse B demonstration is useful for retrospective diagnosis	• Mixture of streptokinase and DNAse is used to lyse clots, thick pus and fibrinous exudates

Option 'b'

STREPTOCOCCI HEMOLYSIN	
Streptolysin O	**Streptolysin S (Serum soluble)**
• Oxygen labile	• Oxygen stable
• Activitiy only on pour plate not on surface	• Responsible for hemolysis seen around streptococcal colonies on surface
• Antigenic protein	• Non Antigenic protein elaborated in presence of serum
• Active only is reduced state	

Note: Lysins similar to streptolysin O are also produced by S. pneumonia (Pneumolysin), Clostridium tetani (tetanolysin), C. perfringens (Q toxin), Bacillus cereus (cereolysin) and Listeria monocytogens (listeriolysin).

Option 'c' *Ref. Textbook of Mirobiology by D.R Arora, 3/e p 228*

Streptokinase (Fibrinolysin)

- Streptococci of group 'A', C and G produce a substance called streptokinase which is actively fibrinolytic for human fibrin. Streptokinase is produced maximally in the early stage of growth.
- Streptokinase converts plasminogen to plasmin, which in turn lyse fibrin.
- Streptokinase facilitates the spread of infection by breaking down fibrin barrier arund the lesion.
- Therapuitically it is used for the treatment of early myocardial infarction and other thromboembolic phenomenon.

Option 'd'

There are three types of pyrogenic exotoxin. Exotoxin A & C are encoded by bacteriophage while type B is coded by chromosome.

18. Ans. (a), (b) Clostridium perfringenes toxin ; Tetasolysin *Ref. Textbook of Microbiology by DR Arora, 1/e p 228*
Already explained

19. Ans. (a) Catalase positivity *Ref. Harrison 18/e, p 1160, 1174, 19/e p 966; Jawetz 25/e, p 200*

Pyoderma (Impetigo)

- Superficial infection of the skin, caused primarily by Group A Streptococci and Staphylococci
- Usual site of infection are face (particularly around nose and mouth) and the legs
- Diagnosis is based on the culture and staining of indeginous lesion.
- Gram positive cocci if catalase positive, than it is most likely to be Staphylococci and if catalase negative than it is Streptococci

20. Ans. (b) i.e. Bacitracin sensitivity *Ref. Greenwood 18/e, p 195*

"Species identification of Pyogenic streptococci is based largely on serological detection of group antigen by immune precipitation or co-agglutination techniques".

An additional test that is useful in the presumptive identification of Str. pyogenes **(Group A Streptococci) is bactracin sensitivity test.** In contrast to most of other streptococci, Str. pyogenes is uniformly sensitive and large inhibition zones are formed around bacitracin discs on blood agar.

- *Str. agulactiae, the other pyogenic streptococci is identified presumptively by the CAMP reaction.*

21. Ans. (c) It is universally susceptible to penicillins *Ref. Harrison 19/e, p 973, 18/e, p 1186*

Enterococci are resistant to penicillin

Disease caused by Enterococci

- UTI (particularly who are on antibiotic treatment)
- Bacterial endocarditis
- Liver abscess, intra-abdominal abscess
- Surgical wound infection.

For more details, refer Ans. 1

22. Ans. (c) Enterococcus *Ref. Ananthanarayan 8/e, p 206, 9/e, p 217; Jawetz 24/e, p 243-244*

GROUP D ENTEROCOCCI	
Enterococci (*E. faecalis*, *E. faecium*)	**Non enterococci (*Strep. bovis*)**
• Able to grow in 6.5% NaCl	• Can't grow
• PYR positive	• PYR negative
• May shows α hemolysis	• Non hemolytic
• Penicillin resistant	• Penicillin susceptible
• Causes UTI and other nosocomial infection	• Causes UTI, endocarditis in patients with GI neoplasm

- Both enterococci and non enterococci group D stereptococci can grow in presence of bile and hydrolyze esculin (bile esculin positive). ... *Jawetz 25/e, p 202, 207, 27/e 226*
- Don't confuse with *Ananthanarayan 9/e, p 218* that non enterococci are inhibited with bile. If question comes go with *Jawetz.*

23. Ans. (c) *Enterococcus faecalis* *Ref. Harrison 19/e, p 971, 18/e, p 1180; CMDT 2014, p 1374*

- Enterococci are catalase negative and grow in chains and above all resistant to cephalosporins.
- Enterococci is a frequent cause of nosocomial bacteremias and many of these enterococci are resistant to vancomycin.
- Enterococcal bacteremias is characteristically seen in ICU in patient taking cephalosporin as antibiotic.
- Enterococci are resistant to all cephalosporins, aminoglycosides and resistant to vancomycin is also becoming common.

Treatment of antibiotic resistant enterococcal infection	
Resistance pattern	***Recommended therapy***
β-lactamase production	Gentamicin plus ampicillin/sulbactam, amoxicillin/clavulanate, imipenem, or vancomycin
β-lactam resistance, but no β-lactamase production	Gentamicin plus vancomycin
High-level gentamicin resistance	***Streptomycin-sensitive isolate:*** Streptomycin plus ampicillin, or vancomycin
	Streptomycin-resistant isolate: Continuous-infusion ampicillin
Vancomycin resistance	Ampicillin plus gentamicin
Vancomycin and β-lactam resistance	No uniformly bactericidal drugs; linezolid (all enterococci) or quinupristin/dalfopristin (*E. faecium* only)

- Three phenotypes of vancomycin resistant enterococci have been identified viz. Van A; Van B; Van C. Van A is associated with high grade resistance to vancomycin and teicoplanin. Van B and Van C are susceptible to teicoplanin.

Other options:

- *Staph. aureus* and coagulase negative staph. are catalase positive.
- *Streptococci viridans* are sensitive to vancomycin.

Remember: Whole streptococci group forms chains while staphylococci group forms bunch similar to grapes.

24. Ans. (a) *Enterococcus faecalis* *Ref. Harrison 19/e, p 974, 18/e, p 1183*

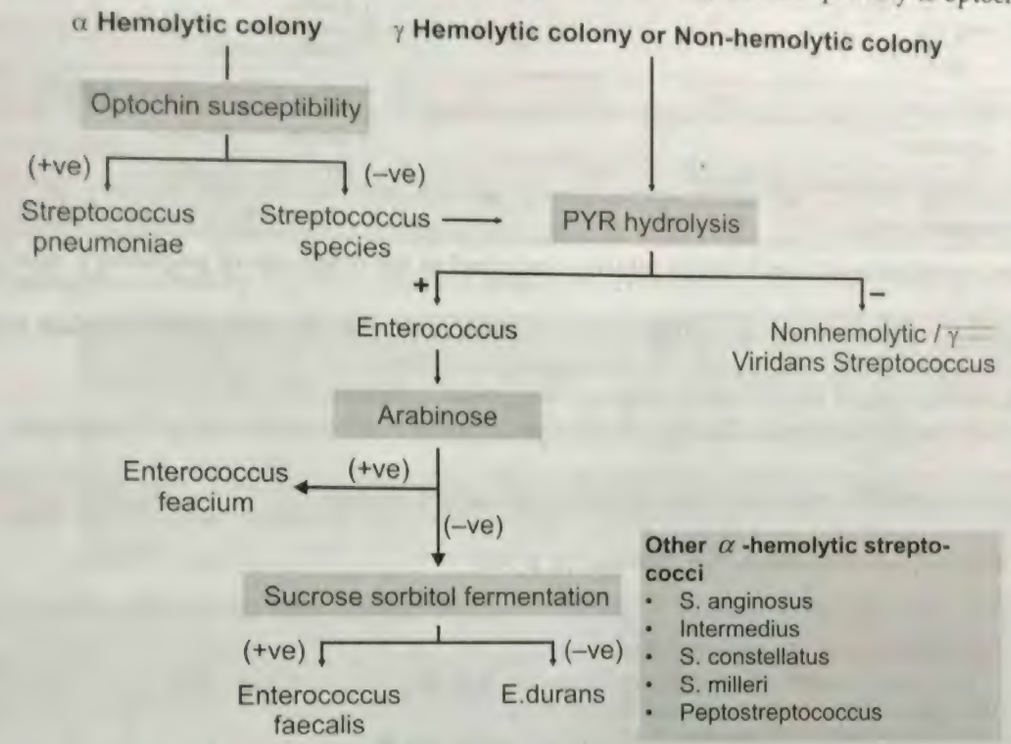
See the following line.

"Unlike streptococci, enterococci are not reliably killed by penicillin or ampicillin alone. Because in vitro testing has shown evidence of synergistic killing by combination of penicillin or ampicillin with an aminoglycoside, combined therapy is recommended for enterococcal endocarditis and meningitis."

- *Enterococci are resistant to all cephalosporins*

25. **Ans. (b) *Enterococcus faecalis*** *Ref. Ananthanarayan 8/e, p 206, 9/e, p 217*
Growth in 6.5% NaCl is characteristic of Enterococci (*E. faecalis, E. faecium, E. durans*).

26. **Ans. (c) Optochin** *Ref. Ananthanarayan 8/e, p219, 9/e, p 222*
Infant is suffering from pneumococcal pneumonia *(Gram-positive a-hemolytic cocci).*
Pneumococci is differentiated from other α hemolytic Gram-positive cocci by its susceptibility to optochin and bile solubiliy.



27. **Ans. (d) *Pneumococcus*** *Ref. Ananthanarayan 8/e, 218-219, 9/e, p 221 - 222*
"Pneumococci is optochin sensitive and forms green colonies on blood agar due to α hemolysis."
Characteristic features of pneumococci:
- Bile soluble, optochin sensitive
- Inulin fermenter
- Capsulated, shows quellung reaction
- Colonies show central umbonation, appear as *draughtsmann* or *carrom coin*
- *MC* causative agent *of lobar pneumonia, adult meningitis, otitis media, sinusitis (orbital cellulitis is complication of sinusitis).*

28. **Ans. (a) Virulence is due to polysaccharide capsule** *Ref. Ananthanarayan 8/e, p 221, 9/e, p 223*
Virulence of pneumococci depends on its capsule, toxin called pneumolysin and autolysin.

Virulence factors of Pneumococci	
Capsule:	• Polysaccharide in nature • Protects against phagocytosis • Type 3 pneumococci has abundant capsular material and is more virulent • Antibody against capsule are type specific and protective.
Pneumolysin:	• Cytotoxin, exerts variety of effects on ciliary cells and PMN. .... *Harrison 18/e, p 1151* • Complement activating and cytotoxic properties • Immunogenic
Autolysin:	• By lysing the bacteria and releasing bacterial components contributes to virulence
IgA1 protease	• Cleaves IgA1 and hence decreases the function of this immunoglobulin.
C-substance and peptidoglycans.	

29. **Ans. (c) Optochin** *Ref. Ananthanarayan 8/e, p 218, 9/e, p 222*
Already explained

30. Ans. (b) Gram-positive cocci in pairs, catalase negative and bile soluble

Ref. Ananthanarayan 8/e, p 206, 9/e, p 222; Jawetz 27/e, p 214

Bacteria which shows α or partial hemolysis includes:

Species	Common disease caused
a. *Strep. viridans*	SABE
S. sanguis,	
S. mutans	Dental caries
b. Pneumococci	Lobar pneumonia, otitis media
c. *S. anginosus*	Pyogenic infections
d. *Enterococcus* including *S.faecalis*	UTI, endocarditis
e. *Peptostreptococus*	Abscesses

- As in quesion, sputum specimen is taken which is required in the diagnosis of respiratory tract infection (e.g. pneumonia).
- *So, it is clear that most probable* answer is Pneumococci which is: dîplococci; bile soluble; catalase negative.
- ***Choice 'a'*** points towards *S. viridans* but it rarely cause pneumonia.
- ***Choice 'c'*** points towards *S. aureus* which shows β hemolysis not α hemolysis.
- *Choice 'd'* points towards *Legionella.* Though it cause pneumonia but it does not shows hemolysis.

Note: All streptococci is catalase negative while staphylococci is catalase positive:

31. Ans. (c) Streptococcus pneumononiae *Ref. Robbins 7/e, p 748*

Remember:

- ***MC*** - infection of pneumococci: Otitis media
- Pneumococci is ***MC*** cause of lobar pneumonia, acute sinusitis, adult meningitis
- ***MC*** cause of bronchopneumonia: Staphylococci
- ***MC*** cause of atypical pneumonia: Mycoplasma pneumoniae.

32. Ans. (a) Pneumococcal *Ref. Harrison 17/e, p 375, 18/e, p 471*

Splenectomy increase risk of following infections	
Bacterial	• Pneumococcal • *H. influenzae* • Some Gram –ve enteric organism
Parasitic	• Babesia
Viral	• Risk of viral infection is not increased

Remember: MC cause of septicemia in asplenic patient - Pneumococci.

33. Ans. (c) Bile solubility *Ref. Harrison 17/e p 865, 18/e p 1151; CMDT 2014, p 262*

This is a classic presentation of pneumococcal pneumonia *(The most common community acquired pneumonia)*

- ***There is confusion between optochin sensitivity and bile solubility as both are unique features of pneumococci.***
  - Bile solubility has got more diagnostic importance as suggested by following lines of *Harrison:*
  - *"More than 98% of pneumococcal isolates are susceptible to ethyl hydrocupreine (optochin) and virtualy all pneumococcal colonies are dissolved by bile salts".* .....*Harrison 17/e 865*
  - Bile solubility of pneumococci is due to the presence of autolytic amidase which are activated by surface active agents (bile salts).
  - ***Inulin fermentation is the other test used to differentiate pneumococci and streptococci.***
  - Pneumococci can ferment inulin, while streptococci don't. This is tested in Hiss's serum water or serum agar slopes

Note: Pneumococci are catalase and oxidase negative gram-positive cocci showing α hemolysis

34. Ans. (c) Pneumococcus *Ref. Harrison 18/e p 1157, 19/e p 951; Nelson 18/e p 2632-2633*

Most common Gram +ve organism causing meningitis : ***Pneumococci***

Most common Gram –ve organism causing meningitis : ***Meningococci***

This holds true for all age group

- Pneumococcal meningitis carries a mortality rate of about 20%.
- In addition 50% of survivors experience acute or chronic complication like deafness, hydrocephalus and mental retardation.
- For diagnosis detection of organism in CSF and/or blood (if clinical symptoms of meningitis are there) is considered highly specific.
- Among adults detection of pneumococcal antigen in urine is also considered highly specific because of low prevalence of nasopharyngeal colonization in adults.

Note: Pneumococcal meningitis can be the primary presenting pneumococcal syndrome or a compli cation of other conditions such as skull fracture, otitis media, mastoid fracture.

35. Ans. (d) i.e. Meningitis caused by S. pneumoniae is milder than others *Ref. Harrison 17/e, p 2627, 18/e p 1156*

Mortality in Bacterial Meningitis	
Cause	**Mortality**
H. Influenzae, Meningococci, Group B streptococci	3-7%
Listeria meningitis	15%
S. pneumococci	20%
E. coli	> 20%

Others options have already been explained.

Note:
- Pneumococci is the most common cause of pneumonia, meningitis, otitis media.
- Otitis media is the most common infection of Pneumococci.

36. Ans. (b) i.e. Optochin *Ref. Greenwood 18/e, p 195*

Pneumococci are distinguished from other a hemolytic stroptococci by their characteristic senstivity to optochin.

Chapter Review

1. **Causative organism of late prosthetic valve endocarditis is:** [AIIMS 97]
   a. Streptococcus viridians
   b. Proteus mirabilis
   c. Beta haemolytic streptococci
   d. Staphylococci
   [Ref. Harrison 18/e, p 1053]

2. **C-Carbohydrate in *Streptococcus hemolyticus* is important for:** [AIIMS 98]
   a. Lancefield classification
   b. Phagocytic inhibition
   c. Toxin production
   d. Hemolysis
   [Ref. Ananthanarayan 9/e, p 209]

3. **Which of the following microorganism is the most common cause of lobar pneumonia:** [MP 06]
   a. *Klebsiella pneumoniae* b. Streptococci
   c. Pneumococci d. Staphylococci
   [Ref. Ananthanarayan 8/e, p 218, 9/e, p 223]

4. **C reactive Protein is:** [AIIMS 91]
   a. Produced by Pneumococcus
   b. A marker of septicemia
   c. Raised in acute inflammation
   d. Low in rheumatoid arthritis
   [Ref. Ananthanarayan 8/e, p 221, 9/e, p 223]

Immunity to Strep pyogenes is type specific and is associated with antibody to M protein. Reinfection occur because of the multiplicity of serotype.

5. ***Streptococcus* causing rheumatic heart is:** [MP 05]
   a. *Streptococcus milleri*
   b. *Streptococcus mutans*
   c. *Streptococcus pyogens*
   d *Streptococcus equimilis*
   [Ref. Ananthanarayan 8/e, p 206, 9/e, p 212]

6. **Draughtsman colonies are seen in:** [UP 05]
   a. *Staphylococcus* b. *Salmonella*
   c. *Pneumococcus* d. *Corynebacterium*
   [Ref. Ananthanarayan 8/e, p 219, 9/e, p 221]

7. ***Streptococcus pneumoniae* true is:** [SGPGI 05]
   a. Vaccine is made from capsular polysaccharide
   b. Vaccine is routinely given to Indian children
   c. Catalase and oxidase positive
   d. Bile insoluble and optochin sensitive
   [Ref. Ananthanarayan 8/e, p 223, 9/e, p 221]

8. **An infant with neonatal meningitis has a positive CAMP test, the causative agent is:** [UP 03]
   a. *Staphylococcus* b. *E.coli*
   c. *Strept agalactiae* d. *Hemophilus*
   [Ref. Ananthanarayan 8/e, p 212, 9/e, p 216]

9. **Causative agent of SABE should be cultured in:** [UP 03]
   a. Nutrient agar b. Blood agar
   c. L. J medium d. Tellurite broth
   [Ref. Harrison 17/e p 792]

10. **Which of the following is not true of diplococcus pneumoniae:** [Kar 01]
    a. Bile solubility b. Optochinin resistance
    c. Causes meningitis d. Possesses capsule
    [Ref. Ananthanarayan 8/e, p 219, 9/e, p 221]

11. **Lymphangitis is caused by:** [AIIMS 95]
    a. *Staphylococcus* b. Streptococci
    c. Pneumococci d. *Neisseria*
    [Ref. CMDT 2014, p 466]

12. **Streptococcal toxic shock syndrome is due to liberation of:**
    a. TSST-1 b. Enterotoxin [Kerala 01]
    c. Pyrogenic exotoxin d. Endotoxin
    [Ref. Ananthanarayan 8/e, p 208, 9/e, p 213]

13. **Most common cause of community acquired pneumonia:** [UP 01]
    a. *Strep. pneumoniae* b. *Kleb. pneumoniae*
    c. *Vibrio cholera* d. *H. influenzae*
    [Ref. Ananthanarayan 9/e, p 223]

14. **True statement about streptococcus faecalis:**
    a. Grows in 6.5% NaCl solution [Kolkata 02]
    b. Are lactose fermenter
    c. Easily destroyed at 60°C for 30° minutes
    d. Classified on the basis of teichoic acid of cell wall
    [Ref. Ananthanarayan 8/e, p 215, 9/e, p 217]

15. **Neonatal meningitis acquired through birth canal is due to:** [TN 02]
    a. *Streptococcus agalactiae*
    b. *S.equisimilus*
    c. *S.pyogenes*
    d. Pnemococci during passage
    [Ref. Ananthanarayan 8/e, p 213, 9/e, p 216]

Answers 1. a. Streptococci... 2. a. Lancefield ... 3. c. Pneumococci 4. c. Raised ... 5. c. Strep ...
6. c. Pneumo ... 7. d. Bile ... 8. c. Strept ... 9. b. Blood ... 10. b. Optochinin ...
11. a, b 12. c. Pyrogenic ... 13. a. *Strep* ... 14. a. Grows ... 15. a. *Streptococc* ...

16. **Which of the following factor is mainly responsible for virulence in *Streptococcus*:** [AIIMS 95]
a. Carbohydrate b. Streptokinase
c. Streptodornase d. M-protein
[Ref. Ananthanarayan, 8/e, p 207, 9/e, p 212]

17. **Streptococcal GN is best diagnosed by:** [PGI 99]
a. ASO titre
b. Anti-DNAse
c. Anti-hyaluronidase
d. Culture
[Ref. Ananthanarayan, 8/e, p 212, 9/e, p 216]

18. **Classification of pathogenic streptococci into group A, B, C, D and G is based on:** [Kar 03]
a. Color of colonies on blood agar
b. Antigenicity of cell wall carbohydrate
c. Presence or absence of capsule
d. Presence or absence of M protein
[Ref. Ananthanarayan, 8/e, p 207, 9/e, p 209]

19. **Impetigo contagiosa is caused by:** [Jharkhand 03]
a. Group A beta hemolytic streptococci
b. *Staphylococcus*
c. *H. influenzae*
d. *Psudomonas* *[Ref. Harrison 17/e, p 799, 18/e, p 1175]*

20. **Most common organism causing sore throat:**
a. *Staphylococcus* b. *Streptococcus* [DNB 2013]
c. *Niesseria* d. *None*
[Ref. Ananthanarayan 9/e, p 213]

21. **Group-A *streptococcus* causes all, except:** [Bihar 04]
a. Scarlet fever b. Erysipelas
c. Impetigo d. Epidermolysis bullosa
[Ref. Ananthanarayan, 8/e, p 209, 9/e, p 210]

Epidermolysis bullosa is a disorder caused by defective keratin, in which the skin and epithelium breaks and blister after minor trauma

22. **Quellung reaction is due to:** [Bihar 04]
a. Capsular swelling
b. Mitochondrial swelling
c. RBC swelling
d. Ribosomal swelling
[Ref. Ananthanarayan, 8/e, p 220, 9/e, p 222]

23. **Virulence or pathogenicity of pneumococcus depends on:** [Kolkata 04]
a. Capsular polysaccharides
b. Cell wall antigen
c. Flagellar antigen
d. Fimbrial antigen
[Ref. Ananthnaryana, 8/e, p 221, 9/e, p 223]

24. **Griffith demonstrated biotransformation with:**
a. *H. influenzae* [UP 03; 04]
b. *E. coli*
c. Proteus
d. Pneumococcus
[Ref. Ananthanarayan, 8/e, p 63, 9/e, p 59]

25. **One of the following infections is caused by anaerobic gram positive cocci:** [AI 95]
a. Puerperal infection
b. Food poisoning
c. Endocarditis
d. Septicemia *[Ref. Ananthanarayan, 9/e, p 266]*

26. **True statements about pneumococcus are all except:** [AI 99]
a. Pneumolysin a thiolactivated toxin, exerts a variety of effects on ciliary and PMN's action
b. Autolysin may contribute to the pathogenesis of pneumococcal disease by lysing bacteria
c. Anticapsular 'antibodies are serotype specific
d. The virulence of pneumococci is dependent only on the production of the capsular polysaccharide
[Ref. Ananthanarayan, 8/e, p 221, 9/e, p 223]

27. **Risk of Pneumococcal meningitis is seen in:**
a. Post-splenectomy patient [AIIMS 99]
b. Patient undergone neurosurgical intervention
c. Patient following cardiac surgery
d. Patient with hypoplasia of lung
[Ref. Harrison, 18/e, p 1155]

28. **False statement regarding pneumococci includes all except:** [DNB 2012]
a. Pneumolysin is the most important virulence factor
b. Quelling reaction is due to C substance
c. IgA_1 protease is an important virulence factor
d. Type I strain is most virulent
[Ref. Greenwood, 18/e, p. 192]

IgA1 Protease is produced by:
- Pneumococci
- Hemophilus influezae
- Neisseria meningitidis

Answers 16. d. M-protein... 17. b, c ... 18. b. Antigeni... 19. a. Group ... 20. b. Streptococcus
21. d. Epider ... 22. a. Capsular ... 23. a. Capsular ... 24. d. *Pneumo* ... 25. a. Puerperal
26. d. The virulence ... 27. a. Post-splenectomy... 28. b. Quelling ...

NEET Pattern Questions

1. **Necrotizing fasciitis is caused by, all except:**
   a. Staphylococcus aureus
   b. Beta haemolytic streptococci
   c. Clostridium perfringens
   d. Pneumococcus

Necrotizing fascitis may be cause by Group A streptococcus or mixed aerobic anaerobic bacteria or may occur as part of gas gangrene caused by Cl. perferingens. Strains of MRSA that produce panton valentine leukociclin (PVL) toxin have also been reported to cause necrotizing fascitis.

[Ref. Harrison, 18/e, p 1068]

2. **Virulence factor of group. A beta haemolytic streptococci:**
   a. Protein M
   b. Protein T
   c. Protein R
   d. Lipotechoic acid

[Ref. Ananthanarayan, 8/e, p 207; 9/e, p 212]

3. **Which group of streptococcus grow at > 60°C:**
   a. A
   b. B
   c. C
   d. D

[Ref. Ananthanarayan, 9/e, p 217]

Enterococcus species are relatively heat resistant can tolerate 60°C for 30 minutes.

4. **False regaruing streptococcurs pyogenes:**
   a. Causes necrotizing fascitis
   b. Beta hemolytic *[Ref. Ananthanarayan, 9/e, p 210]*
   c. M. protein is virulence factor
   d. Resistant to bacitracin

5. **Post-splenectomy patient is in need of—vaccination:**
   a. Pneumococcal
   b. Rota virus
   c. BCG
   d. MMR

[Ref. Harrison, 18/e, p 471]

Pneumoccocal vaccine should be administered to all patients two weeks before electrive splenectomy. Repeat vaccination should be performed five years post splenectomy.

6. **Most common post splenectomy infection is:**
   a. Pneumococcus
   b. Meningococcus
   c. Staphy lococcus
   d. Corynebacterium

[Ref. Harrison, 18/e, p 471]

7. **Streptococcal cell wall polysacharide cross reacts:**
   a. Myocardial muscle
   b. Cardiac valve
   c. Ednocardium
   d. Synovial fluid

[Ref. Ananthanarayan, 9/e, p 212]

Cell wall protein mimics myocardium where as C-carbohydrate (present in middle layer of cell wall) mimics cardiac valves.

8. **Not true regarding the mimcry of Strpyogenes:**
   a. Peptidoglycan and skin antigen
   b. Cell wall protein and myocardium
   c. Hyaluronic acid and synovial fluid
   d. Group A carbohydrate and vascular intima

[Ref. Ananthanarayan, 9/e, p 212

9. **Most common infection caused by streptococcus pneumonia:**
   a. Otitis media
   b. Sore throat
   c. Meningitis
   d. Pneumonia

[Ref. Ananthanarayan, 9/e, p 213

10. **Streptococcus pyogenes shows pathogenicity by al except:**
   a. M protein
   b. Pyrotoxin
   c. Pili
   d. Streptolysin O

[Ref. Ananthanarayan, 9/e, p 213]

11. **Bile esculin agar is used for:**
   a. Group A streptococcus
   b. Group B streptococcus
   c. Group C streptococcus
   d. Enterococcus *[Ref. Jawety, 27/e, p 226]*

Enterococcus grows well in presence of bile and hydrolyze esculin (bile esculin positive).

12. **Dental carries is caused by:**
   a. Streptococcus salivarius
   b. Streptococcus mutans
   c. Streptococcus pyogenes
   d. Streptococcus equisimulus *[Ref. AA, 9/e, p 218]*

13. **Infective endocarditis after tooth extraction is probably due to:**
   a. Streptococcus viridans
   b. Streptococcus pneumoniae
   c. Streptococcus pyogenes
   d. Streptococcus aureus *[Ref. Ananthanarayan, 19/e, p 318]*

Following tooth extraction or other dental procedures, S.sangius produce transient bactremia and get implanted on damaged or prosthehtic value or in a congenitally diseased heart and grow to form vegitation. Thus, under such conditions prophylactic antibiotic cover is advisable before tooth extraction.

14. **Bullous impetigo is caused by:**
   a. Streptococcus
   b. Staphylococcus
   c. Pseudomonas
   d. Clostridium

[Ref. Harrison, 19/e, p 967]

For impetigo also, S.aureus is a prominant cause

15. **Most common cause of cellulitis?:**
   a. Streptococcus
   b. Staphylococcus
   c. Pseudomonas
   d. E. coli

[Ref. Harrison, 19/e, p 967]

Answers 1. **d.** Pneumococcus 2. **a.** Protein M 3. **d.** D 4. **d.** Resistant to bacitracin
5. **a.** Pneumococcal 6. **a.** Pneumococcus 7. **d.** Cardiac valve 8. **d.** Group A carbohydrate and vascular intima
9. **b.** Sore throat 10. **b.** Pyrotoxin 11. **d.** Enterococcus 12. **b.** Streptococcus mutans
13. **a.** Streptococcus viridans 14. **b.** Staphylococcus 15. **a.** Streptococcus

16. Most common organism implicated in causation of necrotizing fascitis:
a. Staphylococcus aureus
b. Streptococcus pyogenes
c. Clostridium perfringens
d. Pneumococcus *[Ref. Harrison, 19/e, p 830]*

17. Crystal violet blood agar is used for which bacteria?
a. Corynebacterium diphtheriae
b. Staph aureus
c. β-hemolytic streptococcus
d. Meningococcus

18. Which streptodornase is most antigenic in human beings:
a. A b. B
c. C d. D
[Ref. Ananthanarayan, 9/e, p 213]

19. Antibiotic used to for sensitivity in identification of streptococcus pyogenes:
a. Bacitracin b. Novobiocin
c. Penicillin d. Optochin
[Ref.Ananthanarayan, 9/e, p 210]

Streptococcus pyogenes is bacitracin sensitive.

20. Not true regarding the mimicry of Strpyogenes:
a. Peptidoglycan and skin antigen
b. Cell wall protein and myocardium
c. Hyaluronic acid and synovial fluid
d. Group A carbohydrate and vascular intima
[Ref. Ananthanarayan, 9/e, p 212]

21. Dental carries is caused by:
a. Streptococcus salivarius
b. Streptococcus mutans
c. Streptococcus pyogenes
d. Streptococcus equisimulus
[Ref. Ananthanarayan, 9/e, p 218]

22. True about streptococcus pyogenes are all except:
a. Causes only localized infection
b. Rheumatic fever is non-supporative complications
c. Erythrogenic toxin causes scarlet fever
d. Glomerulonephritis is due to antigenic cross-reactivity *[Ref. Jawetz, 27/e, p 217]*

Nonsuppurative complications of streptococci:
- Acute rheumatic fever.
- Acute glomerulonephritis.

23. Causative agent of acute rheumatic fever:
a. Group 'A' β-hemolytic streptococcus
b. Group -B β-hemolytic streptococcus
c. Group -C β-hemolytic streptococcus
d. Group -D β-hemolytic streptococcus
[Ref. Ananthanarayan, 9/e, p 210]

24. Lancefield classification of beta hemolytic streptococci is based on:
a. Protein percent
b. Cell wall carbohydrate antigen
c. Cell membrane M protein
d. Hemolytic properties
[Ref. Ananthanarayan, 9/e, p 209]

25. Griffith classification is based on:
a. Cell wall carbohydrate
b. M protein
c. HemolyticR properties
d. None of the above *[Ref. Ananthanarayan, 9/e, p 209]*

26. Streptococcus pyogenes shows pathogenicity by all except:
a. M protein b. Pyrotoxin
c. Pili d. Streptolysin O
[Ref. Ananthanarayan, 9/e, p 212]

27. Culture medium used for streptococcus pneumoniae:
a. Human blood agar b. Sheep blood agar
c. MacConkey's agar d. Deoxycholate agar

28. A chronic alcoholic is presenting with clinical features of meningitis. Most likely organism which will grow on CSF culture:
a. Streptococcus pneumoniae
b. N. meningitidis c. Listeria monocytogenes
d. E. coli *[Ref. Harrison, 19/e, p 885]*

Risk factors for pneumococcal meningitis: sinusitis, otitis media, alcoholism, diabetes, splenectomy, hypogamma globulinemia, CSF rhinorrhea, complement deficiency and head trauma.

29. Bile esculin test is for:
a. Streptococcus pyogenes
b. Streptococcus equinus
c. Enterococcus
d. Streptococcus motons *[Ref. Jawetz, 27/e, p 226]*

30. Heating at 60°C for 30 minute would isolate:
a. Staphylococci
b. Enterococci
c. Micrococci
d. Streptococci
[Ref. Ananthanarayan, 9/e, p 217]

31. Capsule of pneumococcus is:
a. Polypeptide
b. Polysaccharide
c. Lipopolysaccharide
d. Virulence factor *[Ref. Ananthanarayan, 9/e, p 220]*

Answers
16. b. Streptococcus pyogenes
17. c. β-hemolytic streptococcus
18. b. B
19. a. Bacitracin
20. d. Group A carbohydrate and vascular intima
21. b. Streptococcus...
22. a. Causes only localized infection
23. a. Group 'A' β-hemolytic streptococcus
24. b. Cell wall carbohydrate antigen
25. b. M protein
26. None
27. b. Sheep blood agar
28. a. Streptococcus pneumoniae
29. c. Enterococcus
30. b. Enterococci
31. b. Polysaccharide

CHAPTER

9

Neisseria

- Genus consist of *Gram-negative, aerobic, nonmotile, oxidase +ve diplococci* [i.e. grow in pairs].
- Oxidase test is the key test for identifying *Neisseria*.
- Can grow both intracellularly and extracellularly.
- It includes:
  a. *N. meningitidis:* Causative agent of:
     1. **Meningococcal meningitis**
     2. **Purpura fulminans** (Fulminant meningococcemia)
  b. *N. gonorrhoeae:* Causative agent of gonnorhoea
- Nonpathogenic neisseriae grow on ordinary nutrient media, but pathogenic ones require the addition of heated blood (or ascitic fluid) and incubation at 35-37°C in moist atmosphere containing 5-10% CO_2

I

Meningococci:
- Lives commensally in the nasopharynx and is transmitted via close kissing contact.

N. meningitidis [meningococci]	N. gonorrhoeae [gonococci]
• Lens shaped	• Kidney shaped
• Capsulated	• Noncapsulated
• Ferment both glucose and maltose	• Ferment glucose only
• Rarely have plasmid	• Plasmid usually present
• Possess polysaccharide capsule	• Don't possess

N. MENINGITIDIS [MENINGOCOCCI]

- Categorize as β proteobacterium on basis of genome sequencing.
- Natural habitat is human nasopharynx.

I

In India Group A is the most common strain.

Classification

- On *basis* of *capsular polysaccharide* classified into 13 serogroups.
- 5 serogroups A, B, C, W, Y are responsible for most meningococcal disease.

Group A	Epidemic (MC strain in India)
Group B	Both epidemic and outbreak, most prevalent in developed world
Group C	Localized outbreaks
Group Y	Sporadic
Group W	Sporadic, Epidemic in subsaharan Africa

Virulence Factors

Important virulence factors are:

- *Capsular polysaccharide:* Major virulence factor that imparts antiphagocytic and antibacteriocidal properties. Acapsular strains very rarely cause invasive disease.
- *Outer membrane proteins:* Pilli are complex OMP based organelles that facilitates adhesion.
- *Lipoligosaccharide LOS (endotoxin):* Morbidity and mortality of meningococcal bacteremia and meningitis is directly proportional to amount of circulating meningococcal endotoxin.

I

Meningococci:
- Nonpathogenic neisseriae can grow on ordinary media, whereas pathogenic one require addition of heated blood and incubation at 35-37°C.

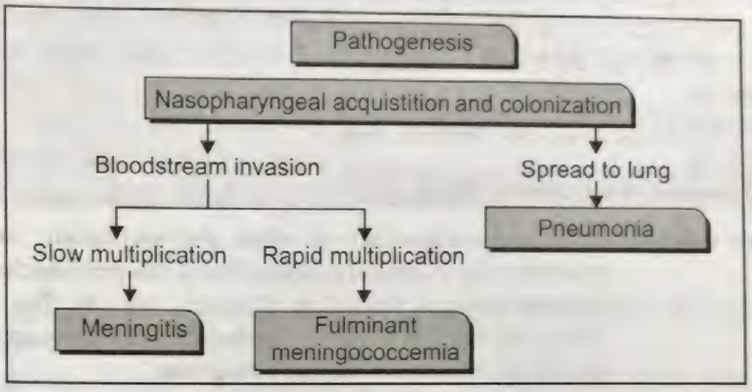
Remember:
- Meningococcal endotoxin is LOS (Harrison 17/e, p 909) not lipopolysaccharide
- Vascular endothelium is particularly sensitive to endotoxin.

I
- Lipid A moiety of lipo-oligosaccharide is the main factor responsible for septic shock syndrome.
- A. Gr(–)ve diplococci with positive urease test suggest diagnosis of meningococci

Pathogenesis

- Meningococci causes invasive disease in susceptible individuals only. Principal determinant of disease susceptibility is age (peak incidence is in first year).
- Deficiency of terminal or alternate complement pathway C_5-C_9 increase risk of meningococcal infection.
- Other factors increasing the risk are cigarette smoking, recent viral respiratory tract infection, overcrowding, infection with mycoplasma species.
- Infants are particularly susceptible to serogroup B. *... Harrison 18/e, p 1212*
- Humans are the only natural hosts for whom meningococci are pathogenic.

I
- Meningitis and for bacteremia are the most common presentation.
- Meningococcal meningitis usually left permanent sequelae like intellectual impairment, cranial nerve deficit



Thus, *meningitis* is result of blood borne dissemination and not direct invasion.
... Harrison 19/e, p 998

Clinical features

a. ***Fulminant meningococcemia [Purpura fulminans]***
   - Most rapid lethal form of septic shock.
   - Differs from other septic shock by *prominence of hemorrhagic skin lesions* (petechiae, purpura) and development of DIC. *Rash* is most distinctive feature.
   - Lab findings include hypoglycemia, acidosis, hypomagnesemia, hypophosphatemia, anemia and coagulopathy.
   - LOS (endotoxin) is responsible for most cases.
   - CSF may be normal and CSF culture may be –ve which is poor prognostic sign.
   - Due to its rapidity acute phase reactant, may remain normal.
   - May progress to ***Waterhouse - Froiderichsen syndrome***–*DIC induced microthrombi, hemorrhage, tissue injury and circulatory collapse.*
   - Endotoxin of Meningococci are capable of producing both generalized and localized Schwartzman reactions. These reactions are involved in the pathogenesis of petechial hemorrhage and Waterhous-Friderichsen syndrome

b. ***Meningitis*** - Common in age between 3 months to 5 years.

c. ***Other manifestation*** - Arthritis, conjuctivitis, urethritis, rarely endocarditis.

Lab diagnosis

- Diagnosis is established by recovering meningococci from sterile body fluids (such as blood, CSF, etc.) or from Petechial aspirate.
- PCR amplification of DNA in buffy coat or CSF samples is the most sensitive method.
- They grow best on *Muller-Hinton* or chocolate agar at 35^0 C in 5 to 10% CO_2.
- ***Thayer Martin media*** is selective media used for culturing throat or nasopharyngeal specimen, as it suppress the compteting oral flora.
- Culture media containing sodium polyethanol sulfonate, which may inhibit meningococcal growth, so should be avoided *... Harrison 19/e, p 999*

Treatment

- 3rd generation cephalosporin such as cefataxime or ceftriaxone is *DOC* for initial therapy.
- Prophylaxis - Rifampin is *DOC* for meningococcal prophylaxis.

Prevention

- *Vaccine* - Quadrivalent meningococcal polysaccharide vaccine [serogroup *A, C, W, Y*].
- *There is no vaccine against serogroup B* as its capsule is nonimmunogenic.
- Vaccine is ineffective in age < 2 years so, given after 2 years.

Indication	Contraindication
• Late complement or properdin deficiency	• Pregnancy
• Asplenia or splenectomy patient	
• Military persons	
• Epidemic areas	
• Pilgrims on Haj	
• Individual travelling to sub-Saharan Africa	

- **Conjugate vaccine:** Poor immunogenicity of plain polysaccharide vaccine has been overcome by chemical conjugation of the polysaccharide to a carrier protein (tetanus toxoid or diptheria toxoid). The rectogenicity of conjugate vaccine is similar to that of plain polysaccharides vaccine. But they yeild better memory response.

I

- Gonococci is the MC cause of PID globally.

N. GONORRHOEAE [GONOCOCCI]

- *MC* cause of PID worldwide [*MC* cause of PID in India is TB].
- *MC* cause of septic arthritis in adult [*MC* joint knee].
- Cause *most severe* type of ophthalmia neonatorum.
- Strains are characterized by auxotyping which recognizes requirement of nutrients

Culture

– More difficult to grow than meningococci	– Essential to provide 5-10% CO_2
– Four types of colonies recognised - T_1 to T_4	– Type 1 and 2 = P^+ and P^{++} respectively.
– Type 3 and 4 = P^- and are avirulent	– They are piliated and virulent.

I

- Both gonococci and meningococci produce IgA_1 protease that inactivales IgA

Biochemical reactions:

- Gonococci ferment only glucose (not maltose)
- Rapid carbohydrate utilisation test (RCUT): This is a rapid, sensitive and specific test for detection of gonococci.

Pathogenic factors

a. **Outer-membrane protein:**
   - Pilli – Piliated strains adhere better to cells and are more virulent.
   - Opacity associated protein (Protein II) – Important for adhesion.
   - Porin (Protein I, III) – *Most abundant* gonococcal surface protein.
   - H.8 Lipoprotein – Excellent target for antibody based diagnostic testing.
   - Transferrin and lactoferrin binding protein.
   - IgA1 protease (also produced by meningococci).

Remember: IgA-1 protease is also produced by S. pneumoniae, H. influenza; some streptococci. ...*Jawetz 27/e, p 283*

I

Gonococcal infection is usually limited to superficial mucosal surfaces lined with columnar epithelium.

b. **Lipooligosaccharide = Endotoxin**
   - Resemble human glycosphingolipid, contributes to the local cytotoxic effects.

c. **Host factors**
   - Defeciency of terminal complement components.

Clinical features

- Mode of infection is almost exclusively veneral except ophthalmia neonatorum.
- *Incubation period:* 2-8 days
- Terminal complement component [C_5-C_9] deficiency predispose to gonococcal infection.
- Higher incidence of gonorrhea occur in blood group B.
- Gonococcal infection in *males*: ***Acute urethritis*** is ***MC*** clinical manifestation of gonorrhea ***in males.***
- Gonococcal infection in *females*: *Cervicitis* is ***MC*** *manifestation*. Adult vagina is resistant to gonococcal infection.
- Gonorrhea in *pregnant woman*: Salpingitis and PID can occur during 1st trim and can cause abortion. In 2nd and 3rd trim, relative impermeability of cervical mucous prevent ascending infection.
- Gonococcal infection in *neonates*: ***MC*** is ophthalmia-neonatorum while *septic arthritis* is ***MC*** manifestation of systemic infection.
- In *children vulvovaginitis* is ***MC*** gonococcal infection.

I

- MC gonococcal manifestation:
  Males: Acute urethritis
  Females: Cervicitis

Diagnosis

- Rapid diagnosis by gram's staining of urethral exudates, organism appear as diplocaci predominantly with in the polymorphs
- Part of sample should be inoculated on ***Thayer Martin*** Media. Detection of gram-negative diplococci or monococci is usually specific.
- It is important to process all samples immediately because gonocci do not tolerate drying.
- If processing is to occur within 6h, transport of specimens may be facilitated by the use of non-nutritive swab transport systems such as *Stuart* or *Amies medium.*
- For longer holding periods culture media with self-contained CO_2 generating systems (such as the JEM BEC or Gono-Pak system may be used).

I

Fitz-Hugh-Curtis Syndrome:

- Peri-hepatic inflammation secondary to transperitoneal spread of gonococci

Treatment

- 3rd generation cephalosporins cefixime and ceftriaxone are *DOC.*
- Penicillin is ***DOC when sensitive (But most isolates are resistant to penicillin)***

Prevention and Control

- Gonorrhea is transmitted from males to females more frequently than in opposite direction.
- Condoms provide effective protection against transmission.

Multiple Choice Questions

Meningococci

1. **The following bacteria are most often associated with acute neonatal meningitis except:** [AI 05]
   a. Escherichia coli
   b. Streptococcus agalactiae
   c. Neisseria meningitidis
   d. Listeria monocytogenes

2. **The following statements about meningococcal meningitis are true except:** [AI 03]
   a. The source of infection is mainly clinical cases
   b. The disease is more common in dry and cold months of the year
   c. Chemoprophylaxis of close contacts of cases is recommended
   d. The vaccines is not affective in children below 2 years of age

3. **Xavier and Yogender stay in the same hostel of same university, Xavier develops infection due to group B meningococcus. After few days Yogender develops infection due to Group - C meningococcus. All the following are true statement except:** [AI 02]
   a. Educate students about meningococcal trans-mission and take preventive measures
   b. Chemoprophylaxis to all against both group B and group C
   c. Vaccine prophylaxis of contacts of Xavier
   d. Vaccine prophylaxis of contacts of Yogendra

4. **Conjugate vaccine are available for the prevention of invasive disease caused by all of the following bacteria except:** [AIIMS 04]
   a. H. influenzae
   b. Strep pneumoniae
   c. Neisseria meningitidis (Group-C)
   d. Neisseria meningitidis (Group-B)

5. **Which of the following is true regarding Neisseria meningitidis infection?** [AIIMS 00]
   a. It is the most common cause of meningitis in children
   b. All strains are uniformly sensitive to sulfonamides
   c. In children less than 2 years the vaccine is not effective
   d. In India sero type B is most common cause

6. **Young female with 3 day fever presents with headache, BP 90/60 mmHg, Heart rate of 114/min, and pin point spots developed distal to BP cuff. Most likely organism is:** [AIIMS May 2013]
   a. Brucella abortus
   b. Brucella suis
   c. N. meningitidis
   d. Staphylococcus aureus

Gonococci

7. **The best site to obtain a swab in asymptomatic gonorrhea is:** [AI 95, 02]
   a. Endocervix
   b. Urethra
   c. Lateral vaginal wall
   d. Posterior fornix

8. **The virulence factor of Neisseria gonorrhoeae includes all of the following except:** [AIIMS 03]
   a. Outer membrane proteins
   b. IgA protease
   c. M protein
   d. Pilli

9. **All are true about Neisseria gonorrhoea except:** [AIIMS 01]
   a. Gram-positive cocci
   b. Causes stricture urethra
   c. Involves seminal vesicles and spreads to epididymis
   d. Drug of choice is ceftriaxone

10. **Which of the following is not true about Neisseria gonorrhoeae?** [AIIMS Nov. 09]
    a. It is an exclusive human pathogen
    b. Some strains may cause disseminated disease
    c. Acute urethritis is most common manifestation in males
    d. All strains are highly sensitive to penicillin

11. **Which is the true statement regarding gonococcal urethritis?** [PGI 06, 00]
    a. Symptoms are more severe in females than in males
    b. Rectum and prostate are resistant to gonococci
    c. Most patients present with symptoms of dysuria
    d. Single dose of ciprofloxacin is effective in treatment
    e. Commonly leads to arthritis

12. **Which deficiency would cause neisseria infection?** [PGI 09]
    a. C9
    b. C8
    c. C7
    d. C6
    e. C5

13. **A man presented with a 3 day H/o lacrimation redness and discharge from left eye. Later on he developed perforation. Discharge from his eye demonstrated gram negative cocci which were oxidase positive. Which of the following can be the probable organism?** [AIIMS May 2013]
    a. Pseudomonas
    b. Acinetobacter
    c. Neissera gonorrhoea
    d. Moraxella catarrhalis

Explanations and References with Illustrative Answers

1. **Ans. (c) *N. meningitidis*** *Ref. Forfar and Arneils - Text book of Paedia, p 319, 1338*

Causes of Meningitis

- Neonatal (< 1 month)
  1. **Bacterial causes:**

Organism	*E. coli* >	*Gp B streptococci* >	Other gram-negative bacilli >	L. monocytogenes
Frequency	34%	30%	8%	6%

Other bacterias are: Staph, other Streptococci, *Pneumococcus, Pseudomonas, Haemophilus, Meningococcus.*

  2. **Viral and protozoal infections:** TORCH, Varicella zoster and HIV.
  3. **Spirochetal and fungal infections**
- **1-11 months:** *N. meningitidis* > *Strep pneumoniae* > *H. influenza*
- **1-20 years:** *N. meningitidis* > *Strep pneumoniae* > *H. influenza*
- More than 20 years : Strep pneumoniae (MC) ..... *Harrison 18/e, p 3410*

2. **Ans. (a) The source of infection is mainly clinical cases** *Ref. Park 22/e, p 156*

Source of infection are carriers not cases.

Meningococcal meningitis or cerebrospinal fever.

- It is caused by *N. meningitides*, a *gram-negative diplococci.*
- Fatality of typical untreated cases is about 80%.
- ***Agent:*** Group A and C and to lesser extent Group B cause major epidemics.
- ***Source:*** Carriers are the most important source of infection. Clinical cases present only a negligible source of infection.
- **Environmental Factors:** Seasonal variation is well established. Outbreaks occur more frequently in the dry and cold months.
- ***Mode of Transmission:*** Mainly by droplet infection.
- ***Incubation Period:*** Usually 3-4 days
- ***Diagnosis:*** Recovering gram-negative diplococci (within pus cells) from sterile body fluids such as CSF, blood
- ***Treatment:*** – Antibiotics save the lives of 95% of patients provided that it is started during the first 2 days of illness.
  – ***Cases:*** [3rd generation cephalosporins are DOC]. ..... *Harrison 17/e, p 912-913, 18/e, p 1216*
  – ***Carriers:*** Rifampicin
- ***Chemoprophylaxis:*** – Rifampicin (the ***DOC*** unless the organism is known to be sensitive to sulfadiazine).
  – It is suggested for close contacts. Mass chemoprophylaxis is restricted to closed and medically supervised communities.
- ***Vaccinization:*** Immunity last for 3 years

3. **Ans. (c) Vaccine prophylaxis of contacts of Xavier** *Ref. Park 22/e, p 157, Jawetz 27/e 288*

For group B meningococcal infection no vaccine is available, as polysaccharide of Group B meningococci is *sialic acid homopolymer* that is poorly immunogenic in humans.

MENINGOCOCCAL VACCINE	
Tetravalent polysaccharide vaccine	**Tetravalent conjugated vaccine**
• Contains purified polyaccharide capsule of A, C, W, Y	• Contains capsular polyaccharide of A, C, W, Y conjugated to diptheria toxoid.
• Poorly immunogenic in children under 18 months	• Induce T-cell dependent response resulting in enhanced primary response among infants too.

• Does'nt confer long lasting immunity (< 3 years)	• Immunity is relatively long lasting
• Nasopharyngeal carriage is not affected	• Reduce carriage
• Use is limited to control of epidemics and in individuals who are at high risk (asplenia, complement deficiencies, laboratory workers, travellers to highly endemic areas, military persons)	• Licensed for routine use in persons 11-55 years of age and for high risk individuals.

4. **Ans. (d) *Neisseria meningitidis* (Group B)** *Ref. Ghai 6/e, p 197 - 198; Harrison 19/e, p 1001*

Already explained

Note: Conjugate vaccine are available for:
a. Hemophilus influenza B.
b. Menigococcal serotypes A, C, Y and W-135.
c. Streptococcal pneumoniae (pneumococcus).

5. **Ans. (a) and (c). It is the most common cause of meningitis in children and In children less than 2 years the vaccine is not effective** *Ref. Park 22/e, p 157; Forfar and Arneil's, p 1338*
   - *MC* cause of neonatal meningitis: *E. coli*
   - *MC* cause of meningitis in age group 1 month to 20 years is *N. meningitidis.*
   - *MC* cause of meningitis > 20 years: *Strep pneumoniae.*
   - Sulphonamides once the mainstay, are not used now due to widespread resistance. *... Ananthanarayan*
   - *MC* serotype is not given in Park or any other book but as convention first written on be taken as the *most common* so group A is *MC* (not group B).
   - Vaccine (not available for group B) is contraindicated in pregnancy and ineffective in children < 2 years.

6. **Ans. (c). i.e. N. meningitidis** *Ref. Harrison 18/e, p 1215, 19/e p 999*
   - The patient here is under state of shock (hypotension, tachycardia) in conjunction with meningitis (headache fever)and rash.
   - Meningococcal meningitis is the most common meningitis complicated by shock.

CLINICAL MANIFESTATION OF MENINGOCOCCAL DISEASE	
Rash	A nonblanching rash (petechial or purpuric) develops in >80% of cases of meningococcal disease. Rashes are initially blanching in nature but within hours become non-blanching. In severe cases large purpuric lesions (purpura fulminons) develops.
Meningitis	Commonly present as fever, vomiting, headache, irritability, petechial or purpuric rash occurs in 2/3 of cases. Headache is seen in adults usually. In upto 40% of cases there are some features of septicemia too.
Septicemia	Can be isolated or as a accomplication of meningitis. The condition may cause death within hours. Purpura fulminans is a specific feature.
Chronic meningococcemia	Present as repeated episodes of petechial rash associated with fever joint pain, arthritis and splenomegaly. The main differential diagnosis is acute rheumatic fever. This condition has been associated with complement deficiently and with inadequate sulfonamide therapy. If left untreated chances of progression to meningococcal septicemia are high.
Post-meningococcal reactive disease	In small proportion of patients, an immune complex disease develops 4-10 days after the onset of meningococcal disease with manifestations that include a maculopapular or vasculitic rash, arthritis, pericarditis and/or polyserosites associated with fever.

7. **Ans. (a) Endocervix** *Ref. Harrison 19/e, p 1007, 18/e, p 1225; Ananthanarayan 8/e, p 229, 9/e, p 232*

Diagnosis Of Gonococcal Infection

SPECIMEN	
In acute gonorrhea	**In chronic gonorrhea**
Endocervical culture—80-90% sensitivity	• Morning drop secretion
Urethral discharge—50% sensitivity	• Exudate after prostatic massage
High vaginal swab are not satisfactory	• Centrifuged deposits of urine when no urethral discharge
Rectal wall swab—if there is history of rectal sex	

Microscopy	Presence of gram-negative diplococci inside polymorphs is suggestive Fluorescent antibody technique, increase the senstivity of microscopy
Transport media	Stuart's or Amies media. For longer holding period culture media with self CO_2 generating system (such as JEMBEC or Gono-pak systems) may be used.
Culture media	Acute → Chocolate agar or Muller-Hinton agar.
	Chronic → Selective media like Thayer-Martin medium.
Serology	CFT: Done in chronic case or with metastatic lesion.
Chemiluminescent DNA Probe	In high-risk patient undergoing screening for STD's.
Blood Culture (e.g. of synovial fluid)	In suspected cases of disseminated infection.

- Preferred method for diagnosis of gonococcal infection in children is a standardized culture, from urethera and cervix However, cervical specimen are not recommended in prepubertial girls unless necessary.

8. **Ans. (c) M protein** *Ref. Harrison 19/e, p 1003, 18/e, p 1220; Ananthanarayan 8/e, p 227, 9/e, p 231*

M protein is the virulence factor of *Strept. pyogenes* not of gonococci.

***Virulence factors of* Neisseria gonorrhea**	
1. Capsular polusaccharide	
2. Outer Membrane Proteins	
• Pilli • Porin (Protein I and III) • IgA1 protease	• Opacity associated protein (Protein II) • Lipoprotein H. 8 • Transferrin and lactoferrin binding protein
3. Lipooligosaccharide (endotoxin)	

9. **Ans. (a) Gram-positive cocci** *Ref. Harrison 19/e, p 1005, 18/e, p 1222*

Gonococcal infection in males	
MC Clinical manifestation	• Acute urethritis.
Major symptoms	• Urethral discharge and dysuria usually without urinary frequency or urgency.
Other features	• Epididymitis (uncommon)
	• Prostatitis (rare).
	• Edema of penis and balanitis.
	• Urethral stricture and Periurethral abscess or fistulae (= Watercan perineum)
	• Inflammation or abscess of Cowper's gland.
	• Seminal vesiculitis
DOC	• 3rd generation cephalosporin—cefixime and ceftriaxone

Remember:
- All cocci are gram-positive except GMC, i.e N. Bonorrhoeae, N. meningococci, N. catarrhalis.
- All important bacilli are gram-negative except **ABC, CML**, i.e. **A**ctinomycetes, **B**acillus, **C**lostridia, **C**orynebacteria, **M**ycobacteria, **L**isteria.
- Testicular involvement is very rare.

10. **Ans. (d) All strains are highly sensitive to penicillin** *Ref. Harrison 19/e, p 1008, 18/e, p 1220-1222*

Let us consider each option

Option 'a'
- *N. gonorrhoeae* is an exclusive human pathogen. The bacteria contains three genome copies per coccal unit ; this polyploidy permits a high level of antigenic variation and the survival of organism

CMDT 2014, p 1414

Option 'b'
- Few strains of *N.gonorrhoea* disseminate through blood stream causing arthritis, skin lesions, endocarditis, meningitis (rare)

Option 'c'

- ***Commonest presentation of gonococcal infection***
  - **Males:** Acute urethritis
  - **Female:** Mucopurulent urinitis

Option 'd'

- **Gonococcal resistance to antimicrobial agents**
  - Gonococci has acquired resistance to sulfonamides, penicillin, fluoroquinolones
  - They acquire resistance either by chromosomal mutation or by acquistation of R factors (plasmid):
    (a) Chromosomal mutation: *Two types of chromosomal mutation have been described:*
    1. Single step drug specific mutation, leading to high level resistance
    2. Mutation involving several chromosomal loci that determine the level as well as pattern of resistance.

    (b) Plasmid borne resistance: Gonococci contains several plasmid. 95% gonococci have a small, cryptic plasmid of unknown function. Two other plasmids contain gene that codes for β lactamase, hence resistance to penicilin. *Tetracycline resistance is also acquired by plasmid.*
  - Alteration in DNA gyrase and topoisomerase IV have been implicated as mechanism of fluoroquinolone resistance.

11. Ans. (c) and (d) Most patients present with symptoms of dysuria and Single dose of ciprofloxacin is effective in treatment *Ref. Harrison 19/e, p 1005, 18/e, p 1222; Ananthanarayan 8/e, p 228, 9/e, p 232*

Gonococcal infection in Females:

- Initial infection involves urethra and cervix uteri.
- Cervicitis is ***MC*** manifestation.
- Vaginal mucosa is resistant due to stratified squamous epithelium but can involve in anestrogenic women (prepubertal, postmenopausal).
- Infection spreads to endometrium, fallopian tube, bartholin gland, peritoneum with perihepatic inflammation (Fitz-Hugh-Curtis syndrome).
- Clinical disease (as a rule, is less severe in women).
- Proctitis occur in both sexes.
- **Disseminated gonococcal infection (DGI) or Arthritis,** occurs in very few patient.
- DGI can cause skin lesion, meningitis, endocarditis, etc.

Treatment: (For both males & females)

- 3rd generation cephalosporin cefixime and ceftriaxone.
- Single dose ciprofloxacin, ofloxacin, levofloxacin, etc. also affective.

Remember: Incubation period of gonococcal infection is 2-8 days.

12. Ans. (a, b, c, d, e) All options are correct *Ref. Harrison 19/e, p 997, 18/e, p 1212*

- Complement is required for bactericidal activity and for efficient opsonophagocytosis. Individuals deficient in any of the late complement components (C5-C9) can not assemble the membrane attack complex (MAC) which is required to kill Neisseria. Thus the incidence of meningoococcal disease is higher among these patients.
- Surprisingly these patients typically develop less severe disease than complement sufficient individuals; and tends to have disease due to uncommon sero-groups.

Remember: Properdin deficienty is another risk factor for Neisseria infection.

13. Ans. (c) Neisseria *Ref. Harrison 18/e, p 1220, 1223, 19/e 1006*

Gram negative oxidase positive bacteria can be none other than neisseria, and here in this case it is N. gonococci.

Occular gonorrhea in adults

- Results from autoinoculation from an infected genital site
- Manifestations range from mild to very severe
- Severe signs include markedly swollen eyelid, chemosis, profuse purulent discharge. Occasionaly there may be corneal ulceration and rarely perforation.
- Diagnosis is confirmed by culture of purulent discharge.

Chapter Review

1. **Differentiation of *Neisseria gonorrhoeae* and *Neisseria meningitidis* is by:** [AI 90]
   a. Glucose fermentation
   b. Maltose fermentation
   c. V. P. reaction
   d. Indol test
   [Ref. Ananthanarayan 8/e, p 230, 9/e, p 231]

2. **Incubation period of gonorrhoea is:** [TN 04]
   a. 1-2 days b. 2-8 days
   c. 8-10 days d. 10-15 days
   [Ref. Ananthanarayan 8/e, p 228, 9/e, p 232]

3. **'Water can perineum' is caused by:** [UP 06]
   a. *E. coli* b. *Enterococcus faecalis*
   c. *Neisseria gonorrhoeae* d. Treponema palladum
   [Ref. Ananthanarayan 8/e, p 228, 9/e, p 232]

4. **A pus culture on chocolate agar shows gram- negative cocci most likely organism is:** [UP 04]
   a. *Haemophils ducreyi*
   b. *Neisseria gonorrhoeae*
   c. *Streptococcus pyogenes*
   d. *Streptococcus pneumoniae*
   [Ref. Ananthanarayan 8/e, p 227, 9/e, p 227]

5. **The vaccine against *N. meningitides* contains:** [ICS 00]
   a. Whole bacteria
   b. Capsular polysaccharide
   c. Somatic '0' antigen
   d. Lipopolysaccharide protein complex
   [Ref. Ananthanarayan 8/e, p 227, 9/e, p 230]

6. **The following are gram-negative cocci except:**
   a. Pneumococci b. Meningococci [Kar 01]
   c. Gonococci d. Veillonella

   Veillonella spp. are a group of small, anaerobic, gram-negative cocci, that are part of the normal flora of the mouth, nasopharynx. Though occasionally isolated in polymicrobial anaerobic infection, they are not associated with any disease.

   [Ref. Ananthanarayan 9/e, p 269]

7. ***Neisseria* infection are associated with:** [CUPGEE 95]
   a. Deficiency of early complements
   b. Deficiency of late complements
   c. There is not such association
   d. Any deficiency can be associated
   [Ref. Ananthanarayan 8/e, p 225, 9/e, p 229]

   Individuals with deficiency in any of the late compliment (C5-C9) cannot assemble membranes attack complex (MAC) needed to kill Neisseria, and are thus prone for infection.

8. **The diagnosis of gonorrohea is established by:**
   a. Complement fixation tests [Orissa 98]
   b. Pili agglutination tests
   c. Hem agglutination tests
   d. All of the above tests
   [Ref. Ananthanarayan 8/e, p 229, 9/e, p 232]

   CFT is useful in diagnosing chronic cases of gonorrhea and gonoccoccal artheritis. Many other serological tests have been attempted, but no test have been found useful for routine diagnosis.

9. **CSF in meningococcal meningitis shows:** [PGI 98]
   a. Gram-positive Diplococci, in pus cells
   b. Gram-negative Diplococci in pus cells
   c. Gram-negative bacilli
   d. Gram-positive bacilli
   [Ref. AA 8/e, p 226, 9/e, p 227]

   Gram-negative diplococci – *Neisseria*
   Gram-positive diplococci – *Pneumococcus*

10. **Gram negative cocci is:** [DNB 2012]
    a. Neisseria
    b. Streptococcal
    c. Staphylococcal
    d. None
    [Ref. Ananthanarayan and Paniker 8/e, p 227]

Answers 1. b. Maltose ... 2. b. 2-8 day 3. c. *Neisseria* ... 4. b. *Neisseria* ... 5. b. Capsular ...
6. a. Pneumococci 7. b. Deficiency ... 8. a. Complement ... 9. b. Gram-negaive... 10. a. Neisseria

NEET Pattern Questions

1. Virulence of gonococci is due to:
a. Pili
b. Endotoxin
c. Exotoxin
d. None
[Ref. Ananthanarayan, 8/e, p 227, 9/e, p 231; Harrison 18/e, p 1220, 1221]

2. Where does gonococci initially infect:
a. Vagina
b. Cervix
c. Uterus
d. Fallopian tubes
[Ref. Ananthanarayan, 9/e, p 232]

3. Meningococci differ from gonococci in that they:
a. Are intra-cellular
b. Prossess a capsule
c. Cause fermentation of glucose
d. Are oxidase positive *[Ref. Ananthanarayan, 9/e, p 227]*

Important difference between meningococci and gonococci is the presence of polysaccharide capsule in meningococci and of plasmids.

4. Waterhouse-Friderichsen syndrome is seen in:
a. Pneumococci
b. N. Meningitidis
c. Pseudomonas
d. Yersinia
[Ref. Ananthanarayan, 9/e, p 229]

Fulminant meningococcimea is called as Waterhouse- Friderichsen syndrome, which is characterized by shock, DIC and multisystem failure.

5. Genus neisseria is:
a. Gram positive diplococci
b. Gram negative diplococci
c. Gram negative coccobacilli
d. Gram positive bacilli *[Ref. Ananthanarayan, 9/e, p 227]*

6. Which of the following can be used for obtaining specimen for isolation of microorganism in laboratory diagnosis?
a. Meningococcal rash
b. Blood in staphylococcal food poisoning
c. Throat swab in Rheumatic fever
d. Blood in post-streptococcal GN
[Ref. Ananthanarayan, 9/e, p 230]

Meningococci can be sometimes demonstrated in petechial lesions by microscopy and culture
- Rheumatic fever and glomerulonephritis requires retrospective diagnosis.

Answers **1. a.** Pili, Endotoxin **2. b.** Cervix **3. b.** Prossess a capsule **4. b.** N. Meningitidis **5. b.** Gram negative diplococci **6. a.** Meningococcal rash

CHAPTER

10

Clostridium

- ***Anaerobic*** obligatory ***Gram positive spore*** bearing bacilli.
- ***Motile*** except ***Cl perferinges*** and ***Cl tetani type VI.***
- *Cl. perfringens* and *Cl. tetani* are found normally in intestine.
- Spores may be:
  - Spherical and terminal *(=Drumstick)* in *Cl. tetani, Cl. tetanomorphum, Cl. sphenoides.*
  - Oval and terminal *(= Tennis racket) Cl. difficle, Cl. tertium, Cl. cochleurum.*
  - Others have either central *(spindle shape)* or sub-terminal (club-shaped) spores.
- Useful ***medium*** for ***Clostridia*** – *Robertson's cooked meat broth.*
- *Important members – Cl. difficle, Cl. perferinges, Cl. tetani, Cl. botulinum.*
- Non-capsulated except *C. perfringens* and *C. butyricum* which are capsulated.

I

Anaerobic Gram(+)ve bacilli motile (except perferingenes) Non-capsulated (except perferingenes and butyricum)

I. CLOSTRIDIUM TETANI

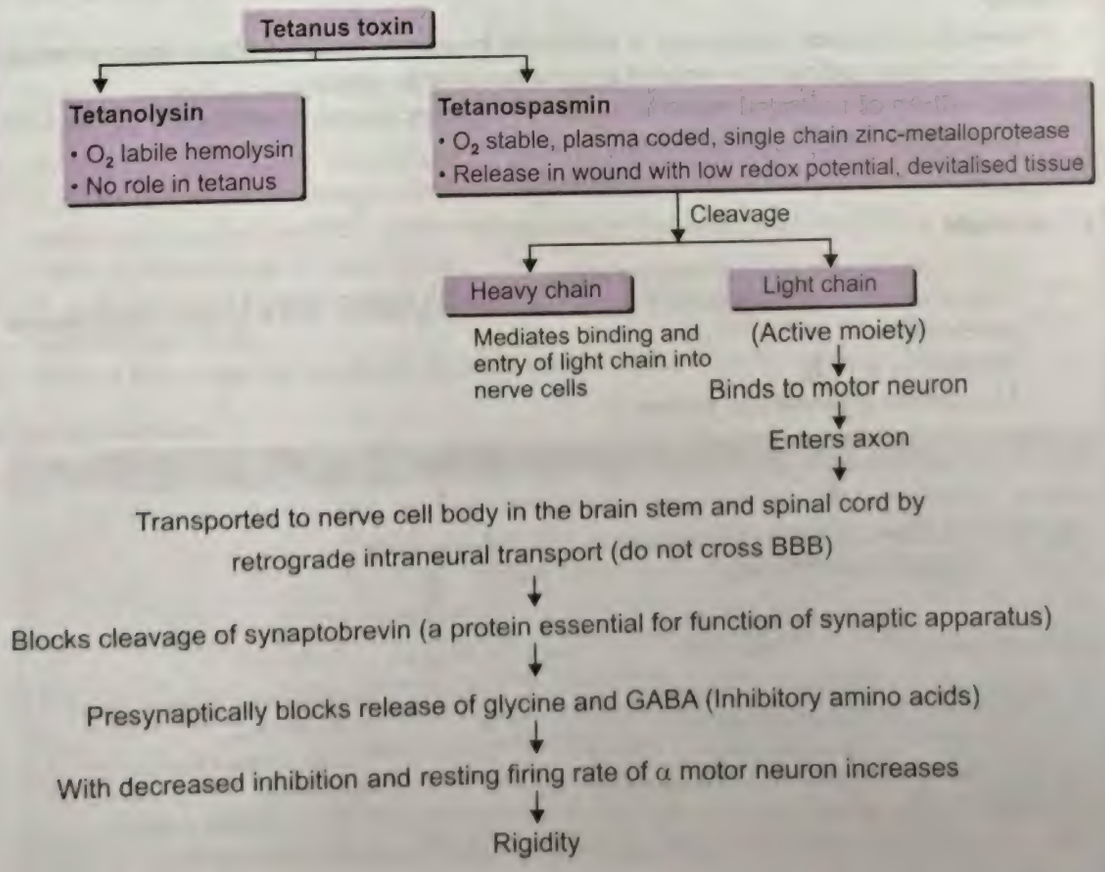
- Causative Agent of Tetanus
- Motile straight slender Gram positive drum stick shaped rod

Culture

- Marked tendency to ***swarm.*** Extremely fine translucent film of growth enables its separation from mixed cultures.
- Show α hemolysis which later develops into β hemolysis.
- It is strict anaerobe and forms surface growth only when O_2 tension is less than 2 mm Hg.
- Spores are resistant to various disinfectants and to boiling for 20 min. Vegetative cells, however, are easily inactivated. Glutaraldehyde is the only sure sporicidal.

I

Spores of C. tetani resist:
- Dry heat at 160°C for 1 hour
- 5% phenol for 2 weeks
- Iodine for few hours



Tetanospasmin affects four areas of nervous system:
- Spinal cord (Mainly)
- Motor end plate in skeletal muscle
- Sympathetic system (resulting symathetic overactivity)
- Brain

Note: Strychinine acts post-synaptically

I
- Gene encoding tetanospasmin is located on plasmid
- In tetanus nerves of head and neck are affected initially as they are short.
- Tetanospasmin exerts its effect on the spinal cord. brainstem, peripheral nerves, at neuromuscular function and directly on muscles

Clinical Manifestations

- *Cl. tetani* has little invasive property. Symptoms are due to the effect of toxin.
- *MC* form of tetanus is *Generalized tetanus in which toxins enter the lymphatics and blood stream.*
- Median time of onset after injury is 7 days.
- ***First symptom*** – *Pain and tingling at the site of inoculation* ....CMDT 2014, p 1382
- ***Early signs*** – Increased tone in masseter muscles called as *trismus or lock jaw,* followed by sequential involvement of nerves of head, trunk and extremities (*Descending tetanus*) since *short nerves are affected earlier. Hands and feet are relatively spared. Sustained contraction of facial muscles results in sinus sardonius.*
- Mentation remains unimpaired.
- ***Deep tendon reflex increase.***
- Autonomic dysfunction (sympathetic increase), sudden cardiac death may occur.
- *Short Incubation period = Grave prognosis.*
- Neonatal tetanus usually occurs as generalized form.
- In local tetanus, only the nerves supplying the affected muscles are involved.
- Cephalic tetanus is a rare form with high mortality, it may occur after head injury or ear infection.

I
Commnest inital symptoms are trismus, muscle pain and stiffness.

Remember: Tetanus patients are not infectious and there is no person to person transmission. Tetanospasmin like botulinum toxin block neurotransmitter release and may produce paralysis, however, this effect is seen in cephalic tetanus only.

I
Pain and tingling at the site of inoculation is the earliest symptom of tetanus.

Diagnosis

- Always clinical, since microscopy is unreliable because it can be isolated from wounds without tetanus and can't be isolated from wounds with tetanus.
- Direct culture of unheated material on blood agar incubated anaerobically is the best method of detecting Cl. tetani. in source (dust, iron).

Treatment

1. *Antitoxin*
- *Best* to give before wound manipulation.
- *Human tetanus immune globulin (TIG)* is *preparation of choice* . T½ = 30 days. It decreases mortality.
- Alternative is IV Ig
- *Management of wound is as follows:*

I
Invasiveness
Cl. tetani: Rarely invasive
Cl. botulinum: Non invasive
Cl. perfringens: Invasive
Cl. novii: Invasive
Cl. histolyticum: Invasive

All wound receive surgical toilet

Wound<6hrs, clean non-penetrating and with negligible tissue damage		Other wounds	
Immunity category	Treatment	Immunity category	Treatment
A	Nothing more required	A	Nothing more required
B	Toxoid one dose	B	Toxoid 1 dose
C	Toxoid one dose	C	Toxoid 1 dose + Human Tetanus Ig
D	Toxoid complete course	D	Toxoid complete course + Human Tetanus Ig

I
Serum antitoxin level ≥0.1 IU/ml are considered protective.

A = Has had a complete course of toxoid or booster dose within past 5 years.
B = Has had a complete course of toxoid or booster dose within past > 5 years and <10 years.
C = Has had a complete course of toxoid or booster dose within past >10 years.
D = Has not had complete course of toxoid or immunity status is unknown.

2. *Antibiotic*

- Penicillin or metronidazole eradicate source of toxin, i.e. vegetative cells.
- Alternative clindamycin or erythromycin.
- It is of no role if given > 6 hours.

Prevention

- Best prevented by ***active immunization.*** Protective level of antitoxin > 0.01 IU/ml serum
- Active immunization – Monovalent Vaccine:
  - 2 dose of purified tetanus toxoid (TT) should be given at interval of 1-2 month
  - Ist booster after 1 year of 2nd dose.
  - 2nd booster after 5 years of 3rd dose.
  - Combined Vaccine - DPT
- Passive immunization – Human tetanus hyperimmunoglobulin (TIG) is *best* prophylactic to use. Toxin already bound to neural tissue is not affected.
- Combined active and passive immunization – Patient is given TIG in one arm and TT in other arm followed by another dose of TT 6 weeks later and third dose 1 year later.

II. CLOSTRIDIUM BOTULINUM

I
Botulinum toxin is charaterized as biothreat level A bological warfare agent
All type can cause hyuman disease but type A, B (both associated with soil) and type E (marine origin) are the most common one.

- Strict anaerobic Gram positive bacillus that causes botulism
- Motile, posess oval and subterminal spores
- It is agent of bioterrorism
- Classified into type A to G based on immunological difference in the toxins.
- Optimal growth temperature is about 35°C but some strains can grow at temperature as low as 5°C

Toxin

- Botulinum toxin is *most potent bacterial toxin known.*
- Produces 8 distinct toxin (A, B, C1, C2, D, E, F, G).
- All toxins except C2 are neurotoxin. C2 is cytotoxin of unknown clinical significance.
- Acts on *peripheral cholinergic nerve endings* including Neuromuscular junctions, post-ganglionic parasympathetic nerve endings and peripheral ganglion, *CNS is not involved. Where it blocks release of Acetylcholine.*
- A, B, E and rarely F cause human disease. Type G (from C. *argentinense*) has been associated with sudden death. ..... *Harrison 19/e, p 987, 18/e, p 1200*
- Type A toxin used for treatment of strabismus, blepharospasm, cervical dystonia.

I
Type E strain: Marine source
Type A &B strain: Soil

Clinical Features

- Cause noninvasive botulism of three types:

1. *Food borne botulism:* – Due to *preformed toxin* of canned food. With incubation period of 18-36 hrs. Nausea vomiting, abdominal pain and *characteristic symmetric cranial perve palsies followed by descending flaccid paralysis occurs.*
   - The source of botulism is usually preserved food, meat and meat products, canned vegitables.
   - Symptoms begin with Cranial Nerve involvement producing diplopia, dysphagia, ptosis, diminished pupillary reflex.
   - Deep tendon reflexes usually decreases but may remain normal, *severe constipation* no fever, no sensory finding, mentally intact.
   - Death is due to respiratory failure.

I
Botulinum toxin differs from other exotoxin in that it is not released during the life of organism.

2. *Wound botulism:* Toxin is produced in wound. It resembles food borne illness except IP is longer (~ 10 days) and gastrointestinal symptoms are lacking.
3. *Infant or intestinal botulism:*
   - Due to ingestion of subterminal spores
   - Toxin is produced inside.
   - Source of infection is usually honey
   - Occur in infant < 6 months.
   - Results in floppy child syndrome.

Remember:
- Botulism is a food poisoning that do not cause diarrhea.
- Infant botulism is most common form of botulism.

Diagnosis

- Demonstration of organism in serum by bioassay in mice is definitive.
- Diagnosis must be considered in patient with symmetric descending paralysis who are mentally intact.

Treatment

- Food borne botulism:
  - No role of antibiotics
  - Guanidine hydrochloride and bivalent antitoxin given
- Wound botulism:
  - Antitoxin
- Infant botulism:
  - Supportive care and human botulism immunoglobulin

III. CLOSTRIDIUM DIFFICILE

- Motile, possess oval and subterminal spores
- Cause CDAD (clostridium diffiicle associated disease)
- *MC* diagnosed diarrheal illness *acquired* in the hospital, which results from ingestion of spores.
- This infection is acquired almost **exclusively** in association with **Antimicrobial use** (*MC clindamycin*).
- **Risk factors** for CDAD: Old age, severe illness, use of electronic rectal thermometer, enteral tube feeding, antacid treatment and gastrointestinal surgery.
- Acquired exogenously and carried in stool of symptomatic and asymptomatic patients.
- Asymptomatic fecal carriage is very common in healthy neonates.
- It releases toxin A (enterotoxin) and toxin B (cytotoxin), both **glucosylate the GTP binding proteins** and disrupt cell cytoskeleton, so fliud is leaked leading to whitish yellow plaque formation over, colon, known as *Pseudomembranous colitis*. Data shows that toxin B is the essential virulence factor.
- *MC* symptom caused by *Cl. difficle* - **Diarrhea**
- *Complication* - Toxic megacolon and sepsis.
- Infants don't develop symptomatic CDAD as they lack receptors for mucosal toxin, which develop later in the life. *.... Harrison 18/e, p 1092*

I

C. difficle is found normally in the faeces of neonates and babies until the age of weaning, but it is not generally found in adults.
C. difficle is the most common cause of hospital acquired diarrhea.

Diagnosis

- ***Diagnosis of CDI is based on a combination of clinical criteria:***
  - 1 (Diarrhea with no other recognized cause
  - 2 Toxin A or B detected is stool
- Demonstrating toxin in feces by its characteristic effect on Hep-2 and human diploid cell culture (Tissue culture) is ***gold standard***. *.... Harrison 19/e, p 859*
- ELISA - Rapid and easy but has less sensitivity.
- Stool culture for clostridium difficle is most sensitive.
- Endoscopy is a rapid diagnostic tool in seriously ill; which demonstrates pseudomembrane.

I

World wide epidemic of fluoroquinolone resistant ribotype 027 Cl. difficile is due to excessive use of fluoroquinolones

Treatment

- *Doc* - Metronidazole
- *Doc even* for relapse is Metronidazole
- If not respond for >48 hours, give vancomycin
- Rifampin, Bacitracin, *Saccharomyces boulardii* or *lactobacillus* GG, colonization with nontoxigenic strain of *Cl. difficle*, anion exchange binding resin, cholestyramine, IV Ig can also given.

I

Treatment of choice for CDAD: Metronidazole.

I

Cl. Welchi: Stormy fermentation.

IV. CLOSTRIDIUM PERFRINGES = CL. WELCHII

- Relatively large with fluent ends.
- Capsulated, non-motile, shows ***Stormy fermentation***
- Causative agent of gas gangrene, enteritis necroticans *(caused by Cl. perfringes type C)*
- Toxigenic as well as invasive.
- Absence of its central or subterminal spore in artificial media or pathological tissue is the characteristic feature.
- Classified on the basis of toxin, they produced.
- It is the ***MC*** clostridial species isolated from tissue infection and bacteremia. *Clostridia* are present in the normal colonic flora, *Clostridia ramosum* is the most abundant and is followed by *C.perfringens*.
- Spores are usually destroyed within five minutes by boiling but those of food poisoning strains of Type A and certain Type C strains resist boiling for 1–3 hours.

Toxins

- Produce twelve distinct toxin. Four major entero toxins are: α, β, ε and ι.
- α *Toxin* = Phospholipase C= lecithinase: Associated with gas gangrene.
  - Hemolytic, Hot-cold variety toxin produced by all *Cl. perfringens* but most abundantly by Type A.
  - Shows Nagler reaction in which zone of opacity is formed where there is no antitoxin.
  - Lecithinase also produced by *Cl. novyi, Cl. bifermentans*, some vibrios.
  - It initiates muscle infection that may progress to gas gangrene.
- β and iota (i) toxin also have lethal and necrotizing properties. Increase capillary permeability.
- θ *(theta) toxin* - Hemolysin, antigenically related to streptolysin O. Also known as ***Perfringolysin O.***
- Also produce neuraminidase, histamine - bursting factor, etc.
- **Nagler Reaction**: When C. perfringens is grown on a medium containing 6% agar, 5% fildes peptic digest of sheep blood, colonies with antitoxin exibit no opacity. In colonies without antitoxin there would be opacity. *This is due to lecithinase effect of α–toxin.*

I

Gas gangrene is caused by:
- C. perfringens
- C. Septicum
- C. novyi type A
- C. histolyticum
- C. sordellil

Clinical Features

It causes following diseases:

1. **Skin and soft tissue infection:**
   - ***a. Gas gangrene = Clostridial myonecrosis:***
     - Most commonly caused by *Cl. perfringes* Type A. Also caused by *Cl. septicum, Cl. novyi, Cl. histolyticum, Cl. sordellil etc.*
     - Characteristic pathology – *Near absence of PMNs despite extensive tissue destruction.*
     - Essential factor - Trauma particularly deep muscle laceration.
     - Incubation period usually short: *10-48 hours* with *Cl. perfringens*, *2-3 days* with *Cl. septicum*, *5-6 days* with *Cl. novyi*.
     - Pain and *crepitus* present and death is due to circulatory failure.

Diagnosis

- Frozen section biopsy of muscle.
- *Gram's staining:*
  - Showing gram-positive bacilli *without spores: Cl. perfringes*
  - Showing *citron* bodies and *boat or leaf shaped* pleomorphic bacilli - *Cl. septicum*
  - Showing large bacilli with oval or subterminal spores - *Cl. novyi.*

Treatment

- Surgery - mainstay of therapy
- *Doc* Clindamycin + penicillin — *Harrison 19/e, p 994*
- Hyperbaric O_2 may also used.

b. Non-traumatic gas gangrene:

- Spontaneous gas gangrene generally occurs via hematogenous seeding of normal muscle with histotoxic Clostridia principally C. perfringens, C. septicum and C. novyi.
- Bacteria reaches blood via GIT (as in colonic malignancy, inflammatory bowel disease, diverticulitis, necrotizing enterocolitis).
- The first symptom of spontaneous gas gangrene is confusion, which is followed by sudden onset of severe pain in absence of trauma. Mortality rate is very high (67-100%) ..... *Harrison 19/e, p 992*

2. Intestinal disorders:

a. Food poisoning:

- *Cl. perfringens* (type A) is 2nd or 3rd ***MC*** cause of food poisoning. It is mediated by Cytotoxin which act on small bowel brush border and induces Ca2+ dependent alteration in permeability.
- Usually caused by cold or warmed up meat dish after 8-24 hours. It is self-limited.
- C. perfingens strains associated with food poisoning possess the gene (CPe) which codes for enterotoxin. ..... *Harrison 19/e, p 991*

b. Enteritis necroticans (Pigbel)

- Caused by β toxin of type C strain.
- Following ingestion of high protein meal with trypsin inhibitors (sweat potato) by host who have limited proteolytic activity in small intestine. Patient present with acute abdominal pain, bloody diarrhea, vomiting, signs of peritonitis.

c. **Necrotizing enterocolitis:**

- Disease resemble *enteritis necroticans* but associated with C. perfringens type A. Affect previously healthy individual.

d. Neurotropenic enterocolitis

I

Cl. perfringens food poisoning is mediated by cytotoxin which induces Ca^{2+} dependent alteration in permeability.

CLOSTRIDIUM SORDELLI

- Rare cause of endometritis and toxic shock syndrome following child birth. .....*CMDT 2014, p 1381*
- Fatal cases of uterine infection following medically induced abortion with mifepristone have been reported.
- Illness occur within 4-5 days of ingestion of mifiperistone.
- Emergent surgical depridement along with C. sordelli susceptible antibiotics is the treatment of choice.

Multiple Choice Questions

CL. PERFRINGENS

1. **The following statements are true regarding *Clostridium perfringens* except:** [AI 05]
   a. It is the commonest cause of gas gangrene
   b. It is normally present in human feces
   c. The principal toxin of *C. perfringens* is the alpha toxin
   d. Gas gangrene producing strains of *C. perfringens* produces heat resistant spores

2. **Regarding gas gangrene one of the following is correct:** [AI 04]
   a. It is due to *Clostridium botulinum* infection
   b. Clostridial species are gram-negative anaerobes forming spores
   c. The clinical features are due to the release of protein endotoxin
   d. Gas is invariably present in the muscle com-partments

3. **Gas gangrene is/are caused by :** [PGI 2011]
   a. Cl. novyi
   b. Cl. septicum
   c. Cl. histolyticum
   d. Cl. perfringens
   e. Cl. tetani

4. **Gastrointestinal enteritis necroticans caused by:** [PGI 07]
   a. Cl. difficile
   b. Cl. perfringens
   c. Botulinum
   d. C. jejuni
   e. Pseudomonas

5. **Nagler's reaction is shown by:** [PGI 00]
   a. Cl. tetani
   b. Cl. botulinum
   c. Cl. perfringens
   d. Cl. septicum

6. **Which species of Clostridium does not cause gangrene?** [AIIMS, May 09]
   a. Cl. perfringens
   b. Cl. histolyticum
   c. C. novyii
   d. C. sporogenes

7. **Regarding *Clostridium perfringens* gas gangrene, all are true, except:** [AIIMS, Nov 10]
   a. Commonest cause of gas gangrene
   b. Naegler reaction positive
   c. Most common toxin is hyaluronidase
   d. Food poisoning strain of *Clostridium perfringes* produced heat resistant spores.

8. **Which of the following regarding Clostridium tetani is false?** [AIIMS, Nov 10]
   a. Soil and intestine of human and animals are the reservoirs
   b. Predominantly seen in dry and winter season
   c. Transmission through contaminated wounds
   d. No herd immunity or seen

9. **True about gas gangrene:** [PGI May 2013]
   a. Underlying skin and muscle are normal
   b. Caused by tetanospasmin toxin
   c. Muscle rigidity and spasm are characteristic
   d. Most common organism implicated is Cl. Perfringes
   e. Passive immunization does not help

CL. TETANI

10. **A person has received complete immunization against tetanus 10 years ago. Now he presents with a clean wound without any lacerations from an injury sustained 2.5 hours ago. He should now be given:** [AI 01]
    a. Full course of tetanus toxoid
    b. Single dose of tetanus toxoid
    c. Human tet. globulin
    d. Human tet. globulin and single dose of toxoid

11. **A 10-year old boy following a road traffic accident presents to the casualty with contaminated wound over the left leg. He has received his complete primary immunization before preschool age and received a booster of DT at school entry age. All of following can be done except:** [AIIMS 01]
    a. Injection of TT
    b. Injection of human antiserum
    c. Broad spectrum antibiotics
    d. Wound debridement and cleaning

12. **The most effective way of preventing tetanus is:** [AIIMS 01]
    a. Surgical debridement and toilet
    b. Hyperbaric oxygen
    c. Antibiotics
    d. Tetanus toxoid

13. **True about clostridium tetani:** [PGI 03]
    a. It is Gram +ve
    b. Drum stick appearance
    c. Grows in aerobic environment
    d. It is Gram -ve
    e. Produces endotoxin

14. **All are true regarding clostridium tetani except:** [AI 2011, AIIMS May 2011]
    a. Produces heat resistant spores
    b. Incubation period 6-11 days
    c. 3 primary doses of vaccine needed for full protection
    d. Man-to-man transmission is seen

OTHERS

15. **True regarding pseudomembranous colitis are all except:** [AI 00]
    a. It is caused by *Clostridium difficle*
    b. The organism is a normal commensal of gut
    c. It is due to production of phospholipase A
    d. It is treated by vancomycin

16. **An 18-year old male presented with acute onset descending paralysis of 3 days duration. There is also a history of blurring of vision for the same dura-tion. On examination, the patient has quadriparesis with areflexia. Both the pupils are non-reactive. The most probable diagnosis is:** [AIIMS 06]
    a. Poliomyelitis
    b. Botulism
    c. Diphtheria
    d. Porphyria

17. **A patient of acute lymphocytic leukemia with fever and neutropenia devlops diarrhea after administration of amoxicillin therapy, which of the following organism is most likely to be the causative agent?** [AIIMS 05]
    a. Salmonella typhi
    b. Clostridium difficle
    c. Clostridium perfringens
    d. Shigella flexneri

18. **The following statements are true regarding botulism except:** [AIIMS 03]
    a. Infant boutlism is caused by ingestion of perfor-med toxin
    b. *Cl. botulinum* A, B, E and F cause human disease
    c. The gene for botulinum toxin is coded by bacteriophage
    d. *Cl. bratii* may cause botulism

19. **Not true about Botulinum toxin:** [PGI 07]
    a. Short life span
    b. Increased acetyl-choline release
    c. Used for treatment in Blepharospasm, static and dyanmic wrinkles
    d. Effective for 3-4 months
    e. Irreversibly decreases Ach in NM junction

20. **Subterminal spores are found in:** [PGI Dec 2008]
    a. Clostridium sordelli
    b. Clostridium sporogenes
    c. Clostridium difficle
    d. Clostridium tertium
    e. Clostridium botulinum

21. **Most important and potential agent that can be used for bioterrosism.** [AI 2011]
    a. Plague
    b. Small pox
    c. TB
    d. Clostridium botulinum

22. **True about treatment of gas gangrene after contaminated road traffic accident:** [PGI 2011]
    a. IV administration of anti-gas gangrene serum
    b. Penicillin
    c. Immediate suturing
    d. Surgical debridement
    e. Irrigation of anti-gas gangrene serum

Explanations and References with Illustrative Answers

1. **Ans. (d) Gas gangrene producing strains of *C. perfringens* produces heat resistant spores**
   Ref. Ananthanarayan 8/e, p 249-55, 9/e, p 254

   "Spores of C. perfringens are heat sensitive":
   - Gas gangrene is caused by *Cl. perfringens* (*MC* by type A), *Cl. novyi, Cl. septicum, Cl. histolyticum* and *C. sordelli*
   - *Most important* toxin for gas gangrene is Alpha toxin = lecithinase which is responsible ***Nagler's Reaction.***
   - *Cl. perfringens* is found in feces and contaminates the skin of perineum, buttocks, thigh.
   - Its spores are used as remote indicator of fecal contamination of water.
   - *Spores are usually destroyed within 5 minutes by boiling but those of food poisoning strains of Type A and certain type C strain resist boiling for several hours.*
   - Spores are destroyed by autoclaving at 121°C for 20 minutes.
   - Spores are resistant to antiseptics and disinfectants in common use.

2. **Ans. (d) Gas is invariably present in the muscle compartments**
   Ref. Ananthanarayan 8/e, p 249, 9/e, p 253; Harrison 19/e, p 992-993, 18/e, p 1206-1207
   - Clostridia are Gram positive (*so produce exotoxin as endotoxin are usually produced by gram negative bacteria*) spore forming anaerobic bacilli.

- *Cl. botulinum* doesn't cause gas gangrene.
- Gas gangrene is characterized by rapid and extensive necrosis of muscle, accompanied by gas formation (clinically as crepitations) and systemic toxicity.

3. **Ans. (a, b and d) Cl. novyi, Cl. septicum, Cl. perfringens** *Ref. Harrison 19/e, p 992; 18/e, p 1206; Ananthanarayan 8/e, p 255, 9/e, p 255*

Clostridium Causing Gas Gangrene		
Common	Less pathogenic	Doubtful
• Cl. perfringens	• Cl. histolyticum	• Cl. bifermentons
• Cl. septicum	• Cl. fallax	• Cl. sporogenes
• Cl. novyi	• Cl. tertium	• Cl. tetani
		• Cl. sordelbii

4. **Ans. (b) *Cl. perfringens*** *Ref. Harrison 19/e, p 991; Ananthanarayan 9/e, p 255*

"Necrotizing enteritis (enteritis necroticians, or pig bel) is caused by β toxin produced by type C strains of C.perfringens following ingestion of a high protein meal in conjunction with trypsin inhibitors by a susceptible host who has limited intestinal proteolytic activity". Source of organism is patient own intestinal flora.

Clinical Features

- Acute abdominal pain, diarrhea, vomiting, shock and peritonitis, 40% of patient die.
- Pathological studies show an acute ulcerative process of the bowel restricted to small intestine.

5. **Ans. (c) Cl. perfringens** *Ref. Ananthanarayan 8/e, p 252, 9/e, p 254*

Naegler's reaction is due to a toxin = lecithinase C = Phospholipidase

So, given by *Cl. perfiringens.*

6. **Ans. (d) C. sporogenes** *Ref. Harrison 19/e, p 992, 18/e, p 1206; Ananthanarayan 8/e, p 255, 9/e, p 255*

Already explained

7. **Ans. (c) Most common toxin is hyaluronidase** *Ref. Ananthanarayan 8/e 249 - 255, 9/e, p 254*

Already explained

8. **Ans. (b) Prodominantly seen in dry and winter season** *Ref. Ananthanarayan 8/e 258, 9/e, p 261; Park 22/e, p 261*

"Tetanus is more common in developing countries where the climate is warm and in rural area where soil is fertile".

Epidemiology of Tetanus

- Natural habitat of Clostridium tetani is soil and dust. Bacilli can be found in intestine of herbivorous animals, e.g. cattle, horses. The spores can survive for years.
- Infection is acquired by contamination of wound with spores.
- ***Sequence of events are:*** introduction of spores; germination, *elaboration of exotoxin* and binding to the receptor.
- It is not transmitted from person to person.

9. **Ans. (d) Most common organism implicated is Cl. Perfringes** *Ref. Harrison 18/e, p 1205-06; 19/e 992*

Gas gangrene

- Rapidly spreading, edematous myonecrosis occurring characterstically in association with wound contamination.
- Clostridium perfringens is the most common organism associated, C. septicum and C. tertium are aerotolerant cause of gas gangrene
- The major C. perfringenes toxin implicated in gas gangrene are α toxin and θ toxin
- In case of C. septicum four toxins have been implicated:
  - α ***toxin*** (lethal, hemolytic, necrotizing activity), β ***toxin*** (DNase) γ ***toxin*** (hyaluronidase) and Δ ***toxin*** (septicolysin)
- Radical surgical debridement along with antibiotic therapy is treatment of choice
- Penicillin plus clindamycin is the preferred antibiotic. If C. tertium is suspected, vancomycin should be used
- Hyperbaric oxygen may be considered after surgery
- Passive immunization with anti gas gangrene serum has not yield any beneficial result and is now not recomended

10. Ans. (b) Single dose of tetanus toxoid *Ref. Park 22/e, p 287*

Management of wound depends on nature of wound and immune status of person.

- All wounds should receive surgical toilet.

Category	Immunization status	Clean wound of < 6 hrs and with negligible tissue damage	Other wounds, e.g. (contaminated wound)
A	Complete immunization within past 5 yrs	Nothing	Nothing
B	Complete immunization within 5-10 years	1 dose of toxoid	Toxoid 1 dose
C	Complete immunization more than 10 years	Toxoid 1 dose	Toxoid 1 dose + Human Tet. Ig
D	Has not had complete immunization or immunity status is unknown	Toxoid complete course	Toxoid complete course + Human Tet Ig

Patient in question falls in category B.

11. Ans. (b) Infection of human antiserum *Ref. Park 22/e, p 287*

"According to table given in previous answer, this child belongs to category B of other wounds since wound is contaminated and child received booster at school entry (so booster > 5 year but < 10 year)."

So, Human antiserum is not needed.

12. Ans. (d) Tetanus toxoid *Ref. Park 21/e, p 287; 22/e, p 287*

Remember: *Best* way of prevention = Active immunization = TT
Best passive immunization = Antitoxin = Human tetanus immunoglobulin.

13. Ans. (a) and (b) It is gram +ve and Drum stick appearance *Ref. Ananthanarayan 8/e, p 250; 9/e, p 253*

- Clostridium is obligatory anaerobic gram-positive spore bearing bacilli.

SPORES OF CLOSTRIDIA		
Spherical and terminal spore (= Drum stick appearance)	**Oval and terminal (= Tennis racket)**	**Central or subterminal**
• Cl. tetani	• Cl. difficle	• Cl. botulinum
• Cl. tetanomorphum	• Cl tertium	• Cl. perfringenes
• C. sphenoides	• Cl. cochleurum	• Cl. septicum • Cl. novyl • Cl. histolyticum • Cl. sporgenes • Cl. sordelli • Cl. chauvoei

14. Ans. (d) i.e. Man-to-man transmission is seen *Ref. Park 21/e, p 285; 22/e p 285*

There is no man to man transmission or a tetanus patient is not infectious.

Other Options

Option 'a'

- Cl. Tetani produce terminal spores which are highly resistant to boiling, cresol, autoclaving for 15 min at 120°C.
- Spores are best destroyed by steam under pressure at 120°C for 20 minutes or by gamma irradiation.

Option 'b'

- I.P. of tetanus is usually 6-10 days however it may be as short as one day or as long as several months.

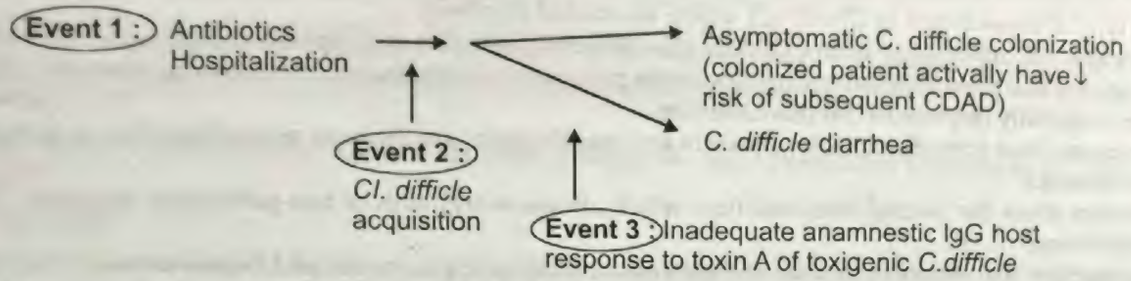
Option 'c'

- Tetanus is best prevented by active immunization with tetanus toxoid.
- The aim is to ensure a life long antitoxin level ≥ 0.01 IU/ml. This can be accomplished either by combined vaccine (DPT) at interval of 48 weeks starting at 6 weeks of age followed by booster at 18 months of age, and a second booster (only DT) at 5-6 years of age
- For monovalent vaccine a primary course of immunizaation consists of two doses of TT given at interval of 1-2 months. This is followed by a booster dose 1 year after the initial dose. Second booster dose is advised after 5 year. Thus, total 4 doses of monovalent vaccine ensure life long protection in an adult.

15. Ans. (c) It is due to production of phospholipase A *Ref. Harrison 19/e, p 858, 18/e, p 1092; Ananthanarayan 8/e, p 263, 7/e, p 265*

"Cl. difficle cause pseudomembranous colitis (PMC) due to the production of toxin A (enterotoxin) and toxin B (cytotoxin) not phospholipase A."

- Toxin A is potent neutrophil chemoattractant and both toxin A and B glucosylate the GTP binding protein of Rho subfamily resulting in disruption of cytoskeleton causing loss of cell shape adherance with consequent fluid leakage.
- Asymptomatic fecal carriage of *Cl. difficle* in healthy neonates is very common. It also colonizes the colon of 3% of healthy adults.
- For *Cl. difficle* associated diarrhea (CDAD) three events are essential: ..... *CMDT*



Diagnosis

- Diagnosis of CAD is based on combination of clinical criteria:
  - Diarrhea (≥ 3 unformed stools per 24 hours for > 2 days)
  - Toxin A or B detected by stool culture or pseudomembrane seen in colon.
- Endoscopy is a rapid diagnostic tool in seriously ill patient with suspected PMC but a negative result does not rule out CDAD.

Treatment

- Discontinue offending antibiotic
- *Drug of Choice*: Metronidazole
- *Drug of Choice* for relapse: Metronidazole
  If not respond > 48 hrs, give Vancomycin.

Note: Patients colonized with *C. difficle* were initially thought to be at high risk for CDI. However four prospective studies have shown that colonized individual actually have a decreased risk of subsequent CDI.

16. Ans. (b) Botulism *Ref. Harrison 19/e, p 988, 18/e, p 1201-1202; Madell's infectious disease 6/e, p 2824*

Diagnosis of boutulism should be considered in patient with symmetric descending paralysis with bilateral cranial neuropathies in addition of following features:

- Absent fever
- Patient remain responsive
- Heart rate normal or slow
- Sensory deficits don't occur except for blurred vision.

Neurologic signs and symptoms of botulism:			
• Dysphagia	• Diplopia	• Dysarthria	• Dry mouth
• Upper limb weakness	• Lower limb weakness	• Burred vision	• Dyspnea

Important Points:

- *Cl. botulinum* produce exotoxin which differs from other exotoxin in that it is produced intracellularly and appears in the medium only on death/autolysis of cell.
- Toxin production is determined by bacteriophage at least in types C and D toxin.
- All toxins are neurotoxin except C_2 which is cytotoxin.
- Toxin acts by blocking production or release of Ach. at synapse and Neuromuscular junction (= parasympatholytic = effect of atropine) so cause constipation, paralysis, etc.
- Human disease is caused by type A, B, E and rarely F.
- *MC* Type of botulism is 'Infant botulism' which is produced by eating of honey containing spores of Cl. botulism which grows and produce toxins.

- *Food borne botulism and wound botulism are produced by preformed toxin.*
- Diagnosis may be confirmed by demonstration of bacillus or toxin in food or feces.
- A retrospective diagnosis may be made by detection of antitoxin in the patient serum but it may not be seen in all cases.

Remember: Polio present with asymmetric descending paralysis.

17. **Ans. (b) Clostridium difficle** *Ref. Greenwood 18/e,p 255; KDT 6/e, p 672 - 73*
    - It is a typical presentation of CDAD (*Cl. difficle* associated diarrhea).
    - *Historically, MC* antibiotics causing CDAD - clindamycin, ampicillin and cephalosporins. *...Harrison 19/e, p 858*
    - Now second and third generation cephalosporins particulary cefotaxine, ceftriaxone cefuroxine and ceftazidine are the agents frequently responsible for this condition. *... Harrison 18/e, p 1091*
    - Fluoroquinolones (ciprofloxacin, levofloxacin and maxifloxacin) are the most recent drug class to be implicated in hospital outbreaks.
    - Antibiotics alters the normal intestinal flora which allows overgrowth of non-pathogenic organism. This is called as superinfection.
    - Superinfection also occurs by *candida albicans*, resistant *staphylococci*, *Proteus* and *Pseudomonas*.
    - Superinfections are common when host defence is compromised as in steroid therapy, leukemia and other malignancies treated with anticancer drugs, AIDS, agranulocytosis, diabetes.

18. **Ans. (a) Infant boutlism is caused by ingestion of preformed toxin** *Ref. Ananthanarayan 8/e, p 262, 7/e, p 263; Harrison 17/e, p 901, 18/e, p 1201*
    - Infant botulism is caused by ingestion of spores which produce toxin in gut.
    - Most of these infants recover with supportive therapy alone.
    - Spores of botulinum are highly resistant to heat, and can withstand 100°C for several hours.

19. **Ans. (a) and (b) Short life span; Increased acetyl-choline release** *Ref. CMDT 2014, p 1383; Jawetz 25/e, p 169*

Botulinum toxin (a zinc metalloprotease) decreases the release of acetylcholine by proteolysis of SNARE proteins. The SNARE proteins are synaptobrevin SNAP-25 and syntaxin. The toxin of C.botulinum types A and E cleaves SNAP-25 while type B toxin cleaves synaptobrevin.

- Though botulinum toxin is the most lethal toxin known (lethal dose 1-2 µg) it is used therapeutically for many conditions.

Therapeutic uses of Botulinum toxin	
Muscular disorders	**Ophthalmic disorders**
Myoclonus	Strabismus
Palatal myoclonus	Lower lid entropion
Focal dystonias	Aquired nystagmus
Tics, tremor	Thyroid ophthalmopathy
Hemi-facial spasm	Dwayne's syndrome
Tourette's syndrome	Oscillopsia
Synkinesis	Apaxia of eyelid opening
Tardive disroders	Hyper-lacrimation
Rigid akinetic syndromes	
Parkinson's progressive supranuclear palsy	
Hallovorden Spatz	
Stiff person syndrome	
Plastic surgery	**Otolaryngology**
Wrinkles	Vocal cord polyps
Masseter hypertrophy	Stuttering
Facial asymmetry (post Bells)	Hypersalivation
Muscle flap paralysis druing healing	
Genitourinary	**Gastroenterology**

Contd...

Contd...

Detrusor - sphincter dyssynergia	Achalasia
Vaginismus	Cricopharyngeal spasms
	Rectal fissures
Rehabilitation Medicine	
Disorders of Painful Muscular Spasm Spasticity: Focal myofascial pain TMJ associated muscle spasm.	

Note: Botulinum toxin once bound leads to permanent dysfunction of that neuron. Recovery (or duration of action) takes 10-20 weeks (usually 3 months) when dysfunctioned nerve terminals are replaced as a result of sprouting.

20. Ans. (a) (b) and (e) Clostridium sordelli, Clostridium sporogenes and Clostridium botulinum *Ref. Ananthanarayan 7/e 250, 9/e, p 252*

Already explained

21. Ans. (d) Clostridum botulinum *Ref. Harrison 17/e, p 1343, 18/e, p 1769*

Cl. botulinum belong to category A of potential bioterrorism agents

Category A bioterrorism agents:

- Anthrax *(Bacillus anthracis)*
- Botulism *(Clostridium botulinum toxin)*
- Plague *(Yersinia pestis)*
- Smallpox *(Variola major)*
- Tularemia *(Francisella tularensis)*
- Viral hemorrhagic fevers

Category A agents are the highest priority pathogens because they:

a. Can be easily disseminated or transmitted from person to person
b. Possess high mortality rates
c. Require special action for public health preparedness.

22. Ans. (a) (b) and (d) IV, Penicillin, Surgical *Ref. Harrison 18/e, p 1205, 1208 - 09, 19/e, p 913 - 14; Ananthanarayan, 8/e, p 256, 9/e, p 255*

Treatment of Gas gangrene

- Emergent Surgical exploration and thorough debridement is most important
- Penicillin G 4 million units IV plus Clindamycin, 600-900 mg IV. If patient is allergic to penicillin then Cefoxitin is used along with Clindamycin.
- Hyperbaric oxygen therapy may be considered after surgery and antibiotics have been initiated.

Note: Closure of traumatic wounds or compound fracture should be delayed for 5-6 days until it is certain that these sites are free of infection.

Chapter Review

1. ***Clostridium* group causes all except:** **[AP 04]**
   a. Acute oesophagitis b. Diarrhea
   c. Local necrosis d. Antibiotic associated changes
   [Ref. Ananthanarayan 9/e, p 251-260]

2. **Botulinum toxin acts on:** **[AI 92]**
   a. Sympathetic system b. Parasympathetic system
   c. Amygdala d. Motor cortex
   [Ref. Ananthanarayan 8/e, p 262, 9/e, p 255]

3. **Swarming growth is seen in which gram-positive bacilli?**
   a. Cl. welchii b. Cl. tetani **[AI 95]**
   c. Proteus d. Bacillus cereus
   [Ref. Ananthanarayan 8/e, p 257, 9/e, p 259]

4. **Best sample for Clostridium botulinum food poisoning is:** **[Delhi 07]**
   a. Stool b. Blood
   c. Left food d. Vomitus
   [Ref. Ananthanarayan 8/e, p 262, 9/e, p 265]

Diagnosis of food borne botulism is confirmed by demonstration of the baullus or the toxin in food or faeces.

5. **Main site of action of tetanus toxin:** **[UP 07]**
   a. Presynaptic terminal of spinal cord
   b. Postsynaptic terminal of spinal cord
   c. Neuromuscular junction
   d. Muscle fibers *[Ref. Ananthanarayan 8/e, p 258, 9/e, p 260]*

6. **Among the toxin produced by botulinum, the non-neurotoxic one is:** **[Kerala 00; AIIMS 92]**
   a. A b. D
   c. Cl. d. C2
   [Ref. Ananthanarayan 8/e, 9/e, p 264]

7. **Drumstick appearence is seen in:** **[Kerala 00, Orissa 00]**
   a. Cl. tetani b. Cl. tetanomorphum
   c. Cl. sphenoids d. All of the above
   [Ref. Ananthanarayan 8/e, p 250, 9/e, p 252]

8. **Which type of Clostridium tetani has no flagella?**
   a. Type 1 b. Type 2 **[AP 08]**
   c. Type 4 d. Type 6
   [Ref. Ananthanarayan 8/e, p 249, 9/e, p 252]

9. **Boxcar appearance is seen in:** **[DNB 2012]**
   a. Clostridium perfringens
   b. Steptococci c. Toxoplasma
   d. Treponema pallidum *[Learn it]*

10. **Oval bulging terminal spores seen in:** **[UP 04; M.P. 03]**
    a. Cl. tertium
    b. Cl. welchii
    c. Cl. perfringens
    d. Cl. histolyticum
    [Ref. Ananthanarayan 8/e, p 250, 9/e, p 252]

11. **Each of the following statements concerning Clostridium perfringens is correct except:** **[SGPGI 04]**
    a. It causes gas gangrene
    b. It causes food poisoning
    c. It produces an exotoxin
    d. It is a gram-negative rod that does'not ferment lactose
    [Ref. Ananthanarayan 8/e, p 251-253 , 9/e, p 253 - 254]

12. **Diagonsis of tetanus is made by:** **[MP 05]**
    a. Culture of bacteria from wound
    b. Clinically
    c. Four fold raise in antibody against tetanus toxin
    d. Gram staining of biopsy from wound
    [Ref. Ananthanarayan 8/e, p 259, 9/e, p 265]

13. **All occurs in botulism except:** **[AIIMS 97]**
    a. Diplopia b. Diarrhea
    c. Dysphagia d. Dysarthria
    [Ref. AA 8/e, p 262, 9/e, p 265]

14. **Non-motile Clostridia is:** **[UP 06]**
    a. Cl. perfringens b. Cl. novyi
    c. Cl. botulism d. Cl. difficle
    [Ref. Ananthanarayan 8/e, p 249, 9/e, p 252]

15. **All of the following statements about Botulism are true except:** **[AI 97]**
    a. Botulism is caused by endotoxin
    b. Honey ingestion causes infant botulism
    c. Constipation is seen
    d. Detection of antitoxin in the serum can aid in diagnosis
    [Ref. AA 8/e, p 261, 9/e, p 265]

16. **Swarming growth on culture is characteristic of which Gram-negative organism?** **[AI 95]**
    a. Clostridium welchii b. Clostridium tetani
    c. Bacillus cereus d. Proteus mirabilis

Swarming growth is shown by:
a. *Cl. tetani, Bacillus cereus*
   – Gram-positive bacilli
b. *Proteus mirabilis* and *Proteus vulgaris*
   – Gram-negative bacilli. *[Ref. AA 8/e, p 280, 9/e, p 261]*

17. **Most common organism, responsible for pseudo membranous colitis is:** **[AI 99]**
    a. Clostridium difficle b. Clostridium botulism
    c. Clostridium bifermentans
    d. Clostridium histolyticum *[Ref. AA 8/e, p 263, 9/e, p 255]*

Answers 1. a. Acute 2. b. Parasympath ... 3. b. Cl.tetani 4. a. Stool 5. a. Presynaptic...
6. b. D 7. d. All of the... 8. d. type 6... 9. a. Clostridium per... 10. a. Cl. tertium...
11. d. It is a gram... 12. b. Clinically... 13. b. Diarrhea... 14. a. Cl. perfering... 15. a. Botulism
16. d. Proteus mirabilis 17. c. Clostridium bifer ...

NEET Pattern Questions

1. **Tetanospasmin encoding genes are locaed on:**
   a. Chromosome b. Plasmid
   c. Both d. Transposon
   [Ref. Ananthanarayan, 9/e, p 260]

2. **Botulinum toxin acts by:**
   a. Enhancing cAMP
   b. Inhibiting dopamine
   c. Inhibiting acetylcholine release
   d. Inhibiting cAMP *[Ref. Ananthanarayan, 9/e, p 264]*

3. **Nagler reaction is shown by:**
   a. Cl. difficle
   b. Cl. perfringens
   c. Cl. tetani
   d. Cl. butryicum *[Ref. Ananthanarayan, 9/e, p 254]*

4. **Botulism is most commonly due to:**
   a. Egg b. Milk
   c. Meat d. Pulses
   [Ref. Ananthanarayan, 9/e, p 264]

5. **Mechanism of action of tetanospasmin:**
   a. Inhibition of GABA release
   b. Inhibition cAMP
   c. Inactivation of Ach receptors
   d. Inhibition of cGMP
   [Ref. Ananthanarayan, 9/e, p 260; Greenwood 18/e p250]

Tetanus toxin prevents the release of neurotransmitter γ-aminobutyric acid in presynaptic cells.

6. **Botulism causes:**
   a. Descending flaccid paralysis
   b. Descendig spastic paralysis
   c. Ascending paralysis
   d. Ascending spastic paralysis
   [Ref. Harrison 18/e, p 201]

Distinctive feature of botulism is symmetrical cranial nerve palsies followed by symmetric descending flaccid paralysis that may progress to respiratory arrest.

7. **Cause of clostridium difficle associated diarrhea.**
   a. Trauma
   b. Dairy products
   c. Fried rice
   d. Antibiotic use *[Ref. Ananthanarayan, 9/e, p 265]*

8. **Most common cause of gas gangrene:**
   a. Cl welchii
   b. Cl septicum
   c. Cl novyi
   d. Cl histolyticum *[Ref. Ananthanarayan, 9/e, p 264]*

Etiologic agents of gas gangrene:
C. perfringenes (= C.welchii) (60% of cases)
C. novyi and C. septum (20–40% of cases)
C. histolyticum

9. **Which of the following is false about Botulism:**
   a. It is a type food poisoning
   b. Botulinum toxin is a potent neruotoxin
   c. It is an infection and not intoxication
   d. The causative organism is clostridium botulinum
   [Ref. Harrison, 19/e, p 260]

10. **Virulence factor for clostridium tetani:**
    a. Fndotoxin
    b. Tetanolysin
    c. Tetanospasmin
    d. Bacteremia
    [Ref. Ananthanarayan, 9/e, p 260]

Tetanospasmin is the main toxin responsible for tetanus.

11. **Spores of clostridium perfringens are located:**
    a. In the middle of cells
    b. At the poles of cells
    c. Between middle and pole of cells
    d. None of the above *[Ref. Ananthanarayan, 9/e, p 252]*

12. **Not true about gas gangrene:**
    a. Most common cause is Cl perfringens
    b. Extensive necrosis of muscles
    c. Cl perfringens produce heat-labile spores
    d. Metronidazole is the drug of choice
    [Ref. Harrison 19/e, p 994]

C. perfringens spores can be heat labile or heat resistant

13. **Tetanus is caused by:**
    a. Gram positive bacilli
    b. Gram negative bacilli
    c. Gram positive cocci
    d. Gram negative cocci
    [Ref. Ananthanarayan, 9/e, p 264]

Answers 1. **b.** Plasmid 2. **c.** Inhibiting acety ... 3. **b.** Cl. perfringens 4. **c.** Meat 5. **a.** Inhibition of...
6. **a.** Descending ... 7. **d.** Antibiotic use 8. **a.** Cl welchii 9. **c.** It is an infection ...
10. **c.** Tetanospasmin 11. **c.** Between middle... 12. **d.** Metronidazole... 13. **a.** Gram positive bacilli

14. Which of following clostridia is non-invasive:
a. Clostridium novyi
b. Clostridium botulinum
c. Clostridium perfringens
d. Clostridium tetani *[Ref. Ananthanarayan, 9/e, p 264]*

15. Most common symptom of tetanus is:
a. Tonic-clonic seizures
b. Hemplegia
c. Lock-jaw
d. Opisthotonus *[Ref. Harrison, 19/e, p 985]*

16. True about Cl perfringens are all except:
a. Invasive as well as toxigenic
b. Alpha toxin is detected by Naegler's reaction
c. Beta toxin is most important in gas gangrene
d. Theta toxin is perfringolysin
[Ref. Ananthanarayan, 9/e, p 255]

17. All clostridia cause myonecrosis except:
a. C septicum
b. C difficile
c. C novyi
d. C welcil
[Ref. Ananthanarayan, 9/e, p 265]

18. Naegler's reaction is due to:
a. Coagulase
b. Hyaluronidase
c. Lecithinase
d. None of the above
[Ref. Ananthanarayan, 9/e, p 255]

Neuglar's reactions is due to α toxin which is phospholipase C

19. Spore rorming anaerobic gram positive bacilli
a. Bacillus Anthracis
b. Clostridia
c. Corynebacterium
d. Peptostreptococcus

Note:
Spore forming aerobic Gram positive bacilli: Bacillus anthracis

Answers **14.** **b.** Clostridium botulinum **15.** **c.** Lock-jaw **16.** **c.** Beta toxin...
17. **b.** C difficile **18.** c. Lecithinase **19.** b. Clostridia

CHAPTER

11

Corynebacterium

Gram-positive nonmotile rods with *high G+C (guanine + cytosine)* content.
It includes: *C. diphtheriae, C. ulcerans* and other coryneform bacteria like *Arcanobacterium, Rhodococcus.*

CORYNEBACTERIUM DIPHTHERIAE (KLEBS-LOEFFLER BACILLUS)

Morphology

- Characteristically ***club shaped*** pleomorphic, noncapsulated, nonsporing non motile bacilli.
- Arranged in pairs, palisades, clusters *(Chinese letter or cuneiform arrangement).*
- Contain Granules known as *Babes Ernst or volutin granules* which are composed of ***polymetaphosphate*** and are ***more strongly gram-positive.***
- On staining with *Loefler's methylene blue,* granules show *metachromatism.*
- ***Special stain to see granules clearly*** – Albert's, Neisser's and Ponder's. Granules are also called as *polar bodies* since they are arranged on poles.

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Corynebacterium
- Club shaped bacteria contains Babes Ernst or volutin granules
- Cesteine-Tellarite blood agar is a selective medium.

Culture

- Enrichment is necessary. Grows best on a blood or serum containing medium at 35-37°C with or without CO_2 enrichment. Optimum pH is 7.2.
- *Selective medium*: ***Cysteine-Tellurite blood agar (grow in 2 days) or Tinsdale*** medium.
- For rapid growth (6-8 hours) - ***Loeffler's serum slope*** used (also used for Mycobacteria TB).
- Mcleod classified *Corynebacterium* into 3 types:

Feature	C. Gravis	C. Intermidius	C. Mitis
• Colony on tellurite	Daisy head	Frog's egg colony	Poached egg colony
• Hemolysis	Variable	Non-hemolytic	Usually hemolytic
• Glycogen and starch fermentation	Positive	Negative	Negative
• Most common complication	Paralytic and hemorrhagic	Hemorrhagic	Obstructive lesion in air passage
• On broth medium	Form pellicle and a granular deposit	No pellicle only granular deposits	Diffuse turbidity

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- Toxigenicity is under the control of phage gene, invasiveness is under the control of bacterial genes.

Diphtheria Toxin

- *Heat Labile single chain three domain Polypeptide exotoxin,* production depends on iron concentration.
- Composed of 2 fragments: Fragment B for binding and fragment A (enzymatic activity) for inactivating elongation factor (EF-2) on nucleus in presence of NAD. Hence, *inhibits protein synthesis.*
- Fragment B binds to host cell membrane proteins CD-9 and heparin binding epidermal growth factor and triggers the entry of toxin into all through receptor mediated endocytosm.
- Toxin is synthesized in precurser form (inactive) in the pseudomembranous lesion.
- Toxin acts both locally and systemically (mainly) while bacilli remain localized. Hence, Diphtheria is *toxemia not bacteremia.*
- Genes coding diptheria toxin are present on corynebacteriophage beta, and a family of closely related corynebacteriophages are responsible for toxigenic conversion of tox- phenotype to tox + phenotype. This is called as ***lysogenic conversion.***
- The strain almost universally used for toxin production is the *'Park William 8' Strain.*
- Exotoxin is also produced by *C. ulcerans, C. pseudotuberculosis.*
- Both tox + and tox–strains are infectious.
- Growth under iron limiting condition leads to optimal expression of diptheria toxin.
- Toxin has a special affinity for myocardium, adrenals nerve endings.

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- Diphtheria toxin is an exotoxin made-up of two fragments which inhibits protein synthesis.
- The standard strain used for toxin production is the Park Williams 8' Strain

Clinical Features

- Cause diphtheria which is localized infection of mucous membrane or skin. It primarily involves respiratory tract.
- The incubation period is 3-4 days, but it can be as short as one day.
- Diphtherias are of following types:
  1. Respiratory Diphtheria
     - *MC* type Tonsillopharyngeal (Faucial)
     - *MC* symptoms: Fever, sore throat and weakness.
     - Also cause malignant or hypertoxic or bull neck appearance
     - Laryngeal involvement leads to obstruction of lower larynx and lower airways.
     - ***Complications of Respiratory Diphtheria***
       - Mechanical complication (Asphyxia) due to membrane.
       - Systemic effects due to toxin, e.g. myocarditis, peripheral polyneuropathy of descending type.
       - *Myocarditis and neuropathy are the most common and most serious systemic complications.* *...Ref CMDT 2014, p 1386*
       - Risk is greater when involves larynx or tracheobronchial tree and in children (because of small airway size).
       - Neurologic complication appear during first or second week of illness and begins with dysphagia.
       - Cardiac damage is permanent while recovery of nerve damage is the rule.
       - 1st muscle involve in paralysis - palatopharynges.
       - Ciliary paralysis occur but not pupillary paralysis, i.e. blurred vision with preserved light reflex.
       - Cause of death is circulatory failure.
  2. **Cutaneous Diphtheria**
     - Punched out ulcers commonly caused by non-toxigenic strains (tox-).
  3. **Invasive Infection**
     - (Rare) Risk factors are pre-existing cardiac abnormality, IV drug abusers, alcoholic cirrhosis.

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- MC type of diphtheria is tonsillopharyngeal
- For treatment initiation, diagnosis is purely clinical
- Elek's precipitation test is a test for toxigenicity

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- The pseudomembrane is caused by diphtheria toxin mediated necrosis of respiratory epithelial layer, producing fibrinous cogulative exudate

Diagnosis

- Diagnosis of respiratory diphtheria is usually clinical while cutaneous diphtheria requires lab confirmation.
- Lab diagnosis can be either by demonstration of organism or demonstration of toxigenicity by *in vivo* or *in vitro* test.

a. Demonstration of organism: – By Gram-staining of throat swab.
– Culture in specified media (Loeffler slant, tellurite plate).

b. Test for toxicigenicity

In vivo	In vitro
• Done on guinea pigs (can be intra-cutaneous or subcutaneous)	• Elek's gel *ppt.* test
	• PCR for detection of toxigene
	• ELISA
	• Immunochromatographic strip assay (fastest, with in hours)

... Jawetz 27/e, p 194

- Matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) is also a reliable tool for rapid diagnosis of potentialy toxigenic corynebacterium species.

Treatment

- Most important element in treatment of respiratory diphtheria is antitoxin however, it does not prevents colonization nor eradicates carrier state.
- Antibiotics *DOC*•Erythromycin or procaine penicillin G. Alternative is Rifampicin or clindamycin.
- Sedatives or hypnotics are contraindicated.
- Glucocorticoids do not reduce the risk of myocarditis or polyneuropathy.

Prevention

- Active immunization by Toxoid is best method to prevent diphtheria. Though active immunization can prevent manifestation of Diphtheria, it cannot prevent carrier stage.
- Active immunization - Combined DPT is used most commonly.
- Pertussis component in DPT increase potency of diphtheria toxoid.
- Toxoid of diphtheria shows Danysz phenomenon and Ehrlich phenomenon.

I

- Schick test is an intradermal test which provide information regarding immune status of patient
- Pertusis component in DPT increase the potency of diphtheria toxoid.

Schick Test

Intradermal test which provide information regarding:

a. Immune status
b. Hypersensitivity to diphtheria toxin.

In one arm toxin is injected, in other arm heat inactivated toxin is injected and following reaction may be seen.

a.	***-ve reaction***	– No reaction in both arm. Suggest patient is immune to diphtheria.
b.	***+ve reaction***	– Red flush of 10-50 mm with in 24-36 hours, reaching its maximum by 4th to 7th day. Control arm shows no change. Patient is susceptible to diphtheria.
c.	***Pseudopositive reaction***	– Red flush equally on both arm, reaction fades very quickly. This is an allergic type of reaction interpreted as Schick's negative.
d.	***Combined reaction***	– Test arm shows +ve, and control arm shows pseudo (+) ve. Dose of vaccine should be reduced.

C. ULCERANS

Transmitted by cow's milk, usually present as pharyngitis and can mimic respiratory diphtheria. Produce a toxin 95% identical to the diphtheria toxin. Transmitted by dog or cat.

C. PSEUDOTUBERCULOSIS (PREISZ NOCARD BACILLUS)

Typically present as suppurative granulamatous lymphadenitis.
Primarily an animal pathogen.

C. MINUTISSIMUM

Cause *Erythrasma* and exhibits coral-red fluorescence under wood's light.

C. PARVUM

Used as immunomodulator.

I

C. Minutissimum cause erythrasma

CO-UREALYTICUM

- Frequent skin colonizer mainly in hospital patients.
- Associated with UTI

DIPHTHEROIDS

- Corynebacterium resembling C.diphtheriae occur as natural commensal in throat, skin, conjunctiva
- Stain more uniformly than diphtheria bacilli, possess few or no metachromatic granules
- Common diphtheroids are C. pseudodephtheriticum (C. hofmanni) and C. xerosis.

a. C. hofmanni
   - Urease positive diphtheroid found in throat
   - Occasionally associated with respiratory tract infection, pneumonia and long abscess
   - It has been reported to cause endocarditis

b. C. xerosis
   - Found in conjunctival sac

Multiple Choice Questions

1. **The following statements are true about DPT vaccine except:** [AI 04]
   a. Aluminium salt has an adjuvant effect
   b. Whole killed bacteria of *Bordetella pertussis* has an adjuvant effect
   c. Presence of acellular pertussis component increases its immunogenicity
   d. Presence of *H. influenza* type B component increases its immunogenicity

2. **Positive Schick's test indicates that person is:** [AI 02]
   a. Immune to diphtheria
   b. Hypersensitive to diphtheria
   c. Susceptible to diphtheria
   d. Carrier of diphtheria

3. **A child presents with a white patch over the tonsils; diagnosis is made by culture in:** [AI 01]
   a. Loeffler medium
   b. LJ medium
   c. Blood agar
   d. Tellurite medium

4. **True about *Corynebacterium diphtheriae* are all except:** [AI 98]
   a. Iron is required for toxin production
   b. Toxin production is responsible for local reaction
   c. Nonsporing, noncapsular and nonmotile
   d. Toxin production is by lysogenic conversion

5. **Ehrlich phenomenon is seen in:** [PGI 2011]
   a. Mycobacterium TB
   b. Proteus
   c. Staphylococcus
   d. Corynebacterium
   e. Mycoplasma

6. **True about corynebacterium diphtheria includes all of the following except:** [AIIMS 07]
   a. Deep invasion is not seen
   b. Elek's test is done for toxigenicity
   c. Metachromatic granules are seen
   d. Toxigenicity is mediated by chromosomal change

7. **Positive Schick's test indicates that the person is:** [AIIMS 07]
   a. Immune to diphtheria
   b. Hypersensitive to diphtheria
   c. Susceptible to diphtheria
   d. Susceptible and hypersensitive to diphtheria

8. **In a completely and adequately immunized child against diphtheria, the throat swab was collected. It showed the presence of C. diphtheriae organisms on Albert staining. These organisms can have one of the following properties on further processing:** [AIIMS 04]
   a. It can grow on potassium tellurite media
   b. It would show a +ve Elek's gel precipitation test
   c. It can be pathogenic to experimental guinea pig
   d. It can produce cytotoxicity in tissue culture

9. **A 12 years old child presents with fever and cervical lymphadenopathy. Oral examination shows a grey membrane on the right tonsil extending to the anterior pillar. Which of the following medium will be ideal for the culture of the throat swab for a rapid identification of the pathogen?** [AIIMS 02, 99]
   a. Nutrient agar
   b. Blood agar
   c. Loeffler's serum slope
   d. LJ medium

10. **Regarding Schick's test which of the following is false:** [AIIMS 00]
    a. Erythematous reaction in both arms indicate hypersensitivity
    b. Positive test means that person is immune to diphtheria
    c. Diphtheria antitoxin is given intradermally
    d. Test done to find out immune status against diphtheria

11. **A child with fever and pharyngitis which of the following investigation should not to be done:** [AIIMS 00]
    a. Widal test
    b. ASO
    c. Throat swab and culture
    d. Chest X-ray

12. **Which of the following is true about diphtheria except:**
    a. Faucial diphtheria is more dangerous than laryngeal diphtheria [PGI 05]
    b. Laryngeal diphtheria mandates tracheostomy
    c. Child is infectious with faucial diphtheria
    d. Myocarditis may be a complication
    e. Palatal paralysis is irreversible

13. **Metachromatic granules are found in:** [PGI 00]
    a. Diphtheria
    b. Mycoplasma
    c. Gardenerella vaginalis
    d. Chlamydia
    e. Staphylococcus

14. **Clinical diphtheria is caused by:** [PGI June 09]
    a. Corn. diphtheria
    b. C. pyogenes
    c. C. ulcerans
    d. Streptococcus pyogens
    e. Pseudodiphtheriticum

15. **False about C. diphtheriae** [AI 2011]
    a. Toxin production is chromosome mediated
    b. Toxic production is phage mediated
    c. Toxic to heart and neuron
    d. Toxin inhibitis protein synthesis

Explanations and References with Illustrative Answers

1. **Ans. (d) Presence of *H. influenza type* B component increases its immunogenicity**

Lets consider each choice one by one.

Option 'a':	Two types of diphtheria toxoid are in use now. *Ref. Ananthanarayan 8/e, p 238* 1. Fluid toxoid (Also known as Formol toxin): Prepared by incubating the toxin with formalin 2. Adsorbed toxoid : Adsorbed toxoid is purified toxoid adsorbed into insoluble aluminium compounds usually aluminium phosphate or less often hydroxide. : Adsorbed toxoid is much more immunogenic than fluid toxoid.
Option 'b':	Pertussis component in DPT (whole killed bacteria of bordetella pertussis) vaccine enhances the potency of the diphtheria toxoid. Most serous complication of DPT is neurological which is primarily due to pertussis component. Duration of immunity after whole cell pertussis vaccination is short lived, with little protection remaining after 10-12 years.
Option 'c':	To reduce complications of whole killed bacteria of pertussis in DPT, acellular vaccine developed. • Protection against pertussis by vaccines correlated best with the production of antibody to pertactin, fimbriae and pertussis toxin. • All acellular pertussis vaccines currently available contain pertussis toxoid. • Acellular vaccine is more immunogenic has less adverse effects. It is given as DTaP. • DTaP has replaced DTP in 1997. DTaP is a tetanus toxoid, reduced diptheria toxoid and acellular pertusis vaccine formulated for adolescent and adults. *.... Harrison 18/e, p 1191*
Option 'd':	Quadriple vaccine of DPT with H. influenza B is available in India but conjugate vaccine (e.g. Hlb vaccine) do not interfere with immunogenicity of simultaneously given other vaccines. *.... Ghai 6/e, p 197*

2. **Ans. (c) Susceptible to diphtheria** *Ref. Ananthanarayan 8/e, p 237, 9/e, p 241; Park 22/e, p 153*

Schick Test

- Intradermal test which provide information regarding:
  a. Immune status,
  b. Hypersensitivity and susceptibility to diphtheria toxin before giving active immunization.
- In one arm toxin is injected (test arm) and in other arm heat inactivated toxin is injected (control arm).

Type of reaction	Observation	Inference
i. Negative reaction	No reaction in both arm (toxin is neutralized by circulating antitoxin)	No susceptibility. No hypersensitivity Patient is immune to diphtheria
ii. Positive reaction	No change in control arm. Red flush in test arm that persist	No hypersensitivity Susceptibility present
iii. Pseudopositive reaction (Schick's negative)	Red flush equally on both arm that fades very quickly	Hypersensitivity present No susceptibility
iv. Combined reaction	Test arm shows positive and control arm shows pseudo- positive reaction	Hypersensitivity present Susceptibility present Dose of vaccine should be reduced

Remember:
- Antitoxin level of 0.01 unit or more per ml of blood is considered as index of immunity.
- Schick test is no longer in use. The level of antitoxin, is assesed by passive hemagglutination or by neutralization in cell culture.

3. **Ans. (a) Loeffler medium** *Ref. Ananthanarayan 8/e, p 233, 9/e, p 237*

In a child with white patch over tonsils, probable diagnosis is diphtheria.

Culture media for corynebacterium are:

i. *Loeffler serum slope:* Growth is very rapid and colonies *seen in 6-8 hr*, before other bacteria grows. It is also used for *M. tuberculosis*. Diphtheria is emergency condition, so Loeffler's slope is preferred media in this child.

ii. *Tellurite blood agar media:* Selective media but growth is delayed and may take about 2 days to appear.

Remember:
- LJ media, is for Mycobacteria TB.
- McLeods and Hoyle's are modified Tellurite media.

Diagnosis of Diphtheria

i. ***Isolation by culture:***
Swabs are inoculated on:
- Loeffler's serum slope (growth is rapid): For rapid diagnosis.
- Tellurite blood agar (growth is delayed but it is particularly important in isolation of bacilli from convalescent, contacts, carriers): Selective media.
- Blood agar (for differentiating staphylococcal and streptococcal pharyngitis).

ii. ***Demonstration of toxicity of isolated strain:***
In vivo test – done by infected broth emulsion of culture subacutaneously (animal will die) and intracutaneously into (animal will not die) guinea pigs.
In vitro test – Elek's gel precipitation test, tissue culture test on agar overlay of cell culture monolayer.
- ELISA
- PCR for detection of toxigene

Control of Diphtheria

i. ***Cases***
- Antitoxin + penicillin or erythromycin ... *Park 22/e, p 152*

ii. ***Carriers***
- Erythromycin

iii. ***Contacts***
a. When primary immunization or booster dose was received within the previous 2 years.
   - No further treatment.
b. When primary immunization or booster dose was received more than 2 years ago.
   - Only a booster dose of diphtheria toxoid.
c. Nonimmunized close contacts:
   - Prophylactic penicillin or erythromycin
   - 1000-2000 units of diphtheria antitoxin
   - Active immunization against diphtheria.

iv. ***Community***
- Only effective control is by active immunization with diphtheria toxoid of all infants with subsequent booster doses every 10 years thereafter.

Remember: Vaccine 'being a toxoid' is not directed against organism and hence immunization does not prevent carrier state which is due to organism not due to toxin

4. Ans. (b) Toxin production is responsible for local reaction *Ref. Ananthanarayan 8/e, p 234, 9/e, p 238-239; Jawetz 27/e, p 194*

"Mechanical complications of diphtheria are due to the membrane while the systemic effects are due to the toxin."
- Toxin acts *mainly systemically though there are partial local effects.*
- It has affinity for myocardium, adrenals and nerve endings.
- Toxin acts by inactivating EF-2 *thus inhibiting protein synthesis.*
- Toxin production is influenced by ***iron*** concentration in the medium. Toxin production is optimal at 0.14 µg/ml and is suppressed at 0.5 µg/ml.
- Toxigenicity of diphtheria bacillus depends on symbiotic bacteriophages, so it shows *lysogenic or phage conversion* i.e. nontoxigenic strain → toxigenic strain by infecting with beta phage.

Remember:
- Corynebacteria are gram-positive, non-acid fast, non-sporing, non-capsulated, non-motile bacteria.
- It contains polar bodies or volutin or Babes-Ernst or metachromatic granules of poly-metaphosphate which are more gram-positive.
- Granules are also stained by Loeffler's methylene blue, Albert's, Neisser's and Ponder's stain.

5. **Ans. (d) Corynebacterium** *Ref. Ananthanarayan 8/e, p 238-239*
   - Diptheria toxin undergoes spontaneous denaturation into toxoid. This toxoid also combines equally with antitoxin. So, in any sample, it would be difficult to estimate the level of toxin as sample will contain a variable amount of toxoid which will vitiate standardisation of antitoxin.
   - Due to the above mentioned issue, two other units for measurement of toxin have been introduced, the Lo and L+ doses.
     - **Lo (Limes nul)** dose of diptheria toxin is the largest amount of toxin that when mixed with one unit of antitoxin and injected subcutaneously into a 250 g guinea pig, will on an average cause no observable reaction.
     - **L+ (Limes tod)** dose of diptheria toxin is the smallest amount of toxin that when mixed with one unit of antitoxin and injected subcutaneously into a 250g guinea pig will on an average kill the animal with in 96 hours.
   - It would be expected that the difference between the L+ dose and Lo dose would be equal to 1 MLD. But when the estimations are actually made, it is found to vary from 10 to 100 MLD or more. This discrepancy is due to the presence of varying amount of toxoid in toxin preparation. This is called as ***Ehrlich phenomenon***.

6. **Ans. (d) Toxigenicity is mediated by chromosomal change** *Ref. Harrison 19/e, p 977, 18/e, p 1188*

   "Corynebacteriophage beta carries the structural gene (tox+) encoding diphtheria toxin and a family of closely related corynebacteriophage are responsible for toxigenic conversion of tox- C.diphtheria to tox+ phenotype."
   - Elek's gel precipitation test is in *vitro test* for toxin detection.

7. **Ans. (d) Susceptible and hypersensitive to diphtheria** *Ref. Ananthanarayan 7/e, p 237*

   Already explained

8. **Ans. (a) It can grow on potassium tellurite media** *Ref. Ananthanarayan 8/e, p 236, 9/e, p 237*

   This child is carrier of diphtheria.
   - Postassium tellurite is selective media for isolation of diphtheria bacillus from convulescent contact, carriers.
   - Other three tests are done for testing virulence only when isolated strain is *C. diphtheriae*.

9. **Ans. (c) Loeffler's serum slope** *Ref. Ananthanarayan 8/e, p 236, 9/e, p 237*

   Already explained

10. **Ans. (b) Positive test means that person is immune to diphtheria** *Ref. Park 22/e, p 153; Ananthanarayan 7/e, p 236*

    Already explained

11. **Ans. (a) Widal test** *Ref. Ananthanarayan 8/e, p 235, 9/e, p 239*

    Widal is test for diagnosing typhoid which is not a cause of pharyngitis, so there is no need to perform widal in this child.

12. **Ans. (a) and (e) Faucial diphtheria is more dangerous than laryngeal diphtheria; and Palatal paralysis is irreversible** *Ref. Dhingra 3/e, p 348; CMDT 2014, p 1386*

    Diphtheria
    - ***Causative agent*** – Corynebacterium diphtheriae (Gram positive bacillus)
    - ***Incubation period*** – 2-6 days
    - ***Depending on site diptheria is classified as:***
      - Nasal diphtheria: Mildest, Toxemia is minimal.
      - Faucial diphtheria (most common) = Nasopharyngeal diphtheria: More severe than nasal diphtheria.
      - Laryngotracheal diphtheria: Most severe, maximum obstructive symptom, tracheostomy may be essential.
    - ***Complications:***
      - Myocarditis – Occurs towards the end of 1st or begining of 2nd week.
      - Peripheral neuropathy of descending type.
      - Renal failure.

Remember:
- Cardiac damage is permanent while recovery of nerve damage is rule.
- Myocarditis and neuropathy are the most common complication.

13. **Ans. (a) Diphtheria** *Ref. Ananthanrayan 8/e, p 232, 9/e, p 236*

Metachromatic Granules

- It is type of ***intracytoplasmic*** inclusions characteristically seen in diphtheria bacilli.
- Also known as *volutin* or *metachromatic* or *Babes-Ernst granules.*
- Strongly ***basophilic*** bodies consist of polymetaphosphate.
- These granules are composed of polymetaphosphate. They are reservoir of energy and phosphate.
- They are more frequent in cells growing under nutritional deficient condition.

Remember:
- Dorset egg media is for M. tuberculosis.
- McConkey is for differentiating between lactose and non-lactose fermenters of Enterobacteriacea.
- Metachromatic granules also seen in B. pertussis.

14. **Ans. (a) Corn. diphtheri** *Ref. Harrison 17/e 890; 893; 19/e 980*

Clinical diphtheria is caused only by *C. diphtheria.*
C. ulcerans can cause diphtheria like lesions (but not diphtheria)

15. **Ans. (a) Toxin production chromosome mediated** *Ref. Ananthanarayan 8/e, p 233, 9/e, p 238; Jawetz 27/e, p 193*
Already explained

Note: Toxicity of corrynebacterium is under control of phage gene, but the invasiveness is under control of bacterial gene.

Chapter Review

1. **Klebloffler's bacillus is:** [RJ 05]
   a. Pneumococci
   b. Gonococci
   c. *Corynebacterium diphtheriae*
   d. Streptococci
   [Ref. Ananthanarayan 8/e, p 232, 9/e, p 237]

2. **Albert's staining is done for:** [RJ 06]
   a. *Clostridium* b. *Corynebacterium*
   c. *Brucella* d. *Pneumococcus*
   [Ref. Ananthanarayan 8/e, p 232, 9/e, p 237]

3. **Spore producing bacteria are all except:** [UP 06]
   a. *Bacillus anthracis*
   b. *Sporosarcina*
   c. *Corynebacterium diphtheriae*
   d. *Clostridium welchii*
   [Ref. Ananthanarayan 8/e, p 232, 9/e, p 237]

4. **Incubation period of diphtheria is:** [Manipal 06]
   a. 2-6 days b. 2-6 hr
   c. 2-6 weeks d. 2-6 months
   [Ref. Ananthanarayan 8/e, p 235, 7/e, p 234]

5. **KLB another name is:** [Jharkhand 04]
   a. *Corynebacterium diphtheriae*
   b. *Corynebacterium psudodiphtheriae*
   c. *Clostridia tetanae*
   d. *Anthrax* *[Ref. Ananthanarayan 8/e, p 232, 9/e, p 236]*

6. **Selective medium for corynebacterium diptheriae is:** [DNB 2011]
   a. Loeffler's serum
   b. Mc Conkey's medium
   c. Cystine-tellurite agar
   d. LJ medium *[Ref. AA 8/e, p 233, 9/e, p 238]*

7. **Culture medium for *Corynebacterium diphtheriae*:** [JIPMER 01]
   a. Loeffler's serum slope
   b. McConkey
   c. Sabarauds agar
   d. Lowenstein-Jensen medium
   [Ref. Ananthanarayan 8/e, p 233, 9/e, p 237]

8. **The type of diphtheria with highest mortality is:** [JIPMER 00]
   a. Pharyngeal b. Nasal
   c. Laryngeal d. Conjunctival
   [Ref. Harrison, 17/e, p 893]

Mildest form of respiratory diphtheria: **Nasal**
Most severe form of respiratory diphtheria: **Laryngeal**

9. **Erythrasma is caused by:** [SRMC 02]
   a. *S. pyogenes*
   b. *Corynebacterium miniutissimum*
   c. *S. aureus*
   d. *Ricketessiae* *[Ref. Ananthanarayan 7/e, p 240, 9/e, p 242]*

Erythrasma is a localized infection of the stratum corneum usually affecting the axilla and grain.
- It is caused by a lipophilic Corynebacterium, C. miniutissimum.
- Bacteria can be grown readily in media containing 20% fetal calf serum.

10. **Literally leather term is used for:** [UP 03]
    a. Anthrax b. Proteus
    c. Diphtheria d. Neisseria
    [Ref. Ananthanarayan 8/e, p 233, 9/e, p 237]

Diphtheria word is derived from diphtherios which means leather. The name is derived from tough, leathery pseudomembrane form in disease.
- Carriers can be detected only by cultural method.
- Swab should be taken from both the nose and throat.

11. **True about diphtheria is:** [SGPGI 03]
    a. Cause cranial nerve palsies in 2nd and 3rd week
    b. Treatment with erythromycin
    c. It is gram-negative organism
    d. Passive immunization is harmful and should not be tried
    [Ref. Harrison 17/e, p 890-893, 18/e, p 1190]

12. **Metachromatic granules are stained by:** [JIPMER 03]
    a. Ponder's stain b. +988Negative stain
    c. Gram's stain d. Leishmans stain
    [Ref. Ananthanarayan 8/e, p 232, 9/e, p 237]

13. **One unit of diphtheria antitoxin was defined as the smallest amount of antitoxin required to neutrilize:** [SGPGI 04]
    a. 100 MLD of toxin b. 200 MLD of toxin
    c. 300 MLD of toxin d. 400 MLD of toxin
    [Ref. Ananthanarayan 8/e, p 238, 7/e, p 238]

Answers 1. c. Coryne... 2. b. Coryne ... 3. c. Coryne... 4. a. 2-6 days 5. a. Corynebac...
6. c. Cystine... 7. a. Loefflers ... 8. c. Laryngeal... 9. b. Corynebac... 10. c. Diptheria...
11. b. Treatment... 12. a. Ponder's... 13. a. 100 MLD...

14. Toxigenicity of C. diphtheria is determined by:
a. Elek's gel ppt test **[Bihar 04]**
b. Aselole's reaction
c. Nagler's reaction
d. All *[Ref. Ananthanarayan 8/e, p 236, 9/e, p 240]*

15. True statement regarding diphtheria is: **[AI 97]**
a. It can be diagnosed by demonstration of antibodies by ELISA
b. Immunization prevents carriers state
c. Treatment of contacts is not indicated
d. Iron has critical value in the production of toxin
[Ref. Ananthanarayan 8/e, p 235-236, 9/e, p 240-241]

16. Investigation of choice of diphtheria carrier is:
a. Throat swab culture **[AIIMS 97]**
b. Gram's stain
c. Albert's stain
d. Ziel-Neelsen stain *[Ref. Park 22/e, p 151]*

Carriers can be detected only by culture method
Swab should be taken from both the nose and throat

17. All are true regarding diphtheria toxin except:
a. Toxin blocks elongation of protein **[AIIMS 97]**
b. Beta lysogenic strain produces toxin
c. Iron is critical for toxin production
d. Toxin is necessary for local wound production
[Ref. Ananthanarayan 8/e, p 235, 9/e, p 238]

18. Corynebacterium diphtheriae can be grown within 6-8 hours on: **[PGI 98]**
a. Potassium tellurite media with iron
b. McConkey's agar
c. Dorset egg medium
d. Loeffler's serum slope
[Ref. Ananthanarayan 8/e, p 232, 9/e, p 237]

Answers **14.** a. Elek's... **15.** d Iron has critical value in the production of toxin **16.** a. Throat swab culture
17. d. Toxin... **18.** d. Loeffler's...

NEET Pattern Questions

1. **True about corynebacterium diphtheriae:**
   a. All types produce toxin
   b. Toxin production is dependent upon critical concentration of iron
   c. Heat stable toxin
   d. Inhibit cAMP *[Ref. Ananthanarayan, 9/e, p 239]*

> Optium lonc. of iron for toxin production is 0.1 mg/l while a concentration of 0.5 mg/l inhibits toxin formation

2. **Lysogenic conversion is seen:**
   a. Diphtheria b. Salmonella
   c. Staphylococcus d. E coli
   [Ref. Ananthanarayan, 8/e, p 234; 9/e, p 238]

3. **A child come with fever, cold, cough, membrane over tonsils; nasal swab is taken, culture should be done on which medium for earliest diagnosis:**
   a. Loffelers serum slop b. L.J. media
   c. MC Conkey's Agar d. Citrate media
   [Ref. Ananthanarayan, 8/e, p 233; 9/e, p 237]

4. **Commonest complication of diptheria:**
   a. Myocarditis b. Neuropathy
   c. Endocarditis d. Pericarditis
   [Ref. CMDT 2014, p 1386]

5. **C. diphtheriae is also called as:**
   a. Koch's bacillus b. Roux bacillus
   c. Klebs-loeffler bacillus d. Yersin bacillus
   [Ref. Ananthanarayan, 9/e, p 236]

> Dipthera bacillus was first observed by Klebs and was first cultivated by Loeffler, so the name Klebs Loeffler bacillus.

6. **Receptor for diphtheria toxin lies at:**
   a. Cell membrane b. Mucous membrane
   c. Nucleus d. None *[Ref. Jawetz, 27/e, p 193]*

> B subunit of diphtheria toxin binds to host cell membrane protein CD-9 and HB-EGF which triggers the entry of A subunit into the cell through endocytosis.

7. **Schick test is for:**
   a. Susceptibility to diphteria
   b. Susceptibility to tetanus
   c. Susceptibility to scarler fever
   d. Susceptibility to RF *[Ref. Ananthanarayan 9/e, p 241]*

> The test is no longer used due to availability of effective and safer toxoid preparation.

8. **Confirmatory tests for C. diphtheriae:**
   a. Serological tests
   b. Isolation in selective media
   c. Tests for toxin *[Ref. Ananthanarayan, 8/e, p 240]*

9. **True about diphtheria toxin:**
   a. Heat stable
   b. Acts through cGMP
   c. Consists of three polypeptides
   d. Special affinity for brain *[Ref. Javetz, 27/e, p 193]*

10. **True about corynebacterium diphtheriae:**
    a. Schick test is done for resistance
    b. Gram positive organism
    c. Schick test is an intramuscular test
    d. Most important treatment is antibiotic
    [Ref. Ananthanarayan, 9/e, p 237]

11. **Earliest growth of diphtheria is detect on which media:**
    a. Potassium tellurite media with iron
    b. McConkey's agar
    c. Dorset egg medium
    d. Loeffler's serum slope *[Ref. Ananthanarayan, 9/e, p 236]*

12. **Chinese letter configuration is seen in:**
    a. Cl tetani b. Cl perfringens
    c. Strept salivarius d. C diphtheriae
    [Ref. Ananthanarayan, 9/e, p 237]

13. **Most dangerous type of diptheria:**
    a. Facial b. Laryngeal
    c. Nasal d. Cutaneous

14. **Not true about corynebacterium hormannii:**
    a. A diphtheroid
    b. Non-pathogenic saprophyte
    c. Toxigenic
    d. Also known as C pseudodiphthericum
    [Ref. Ananthanarayan, 9/e, p 242, Greenwood 18/e, p 204]

15. **Corynebacterum other than diphtheriae carrying toxin:**
    a. Corynebacterium ulcerans
    b. Corynebacterium xerosis
    c. Corynebacterium striatum
    d. Corynebacterium urealyticum
    [Ref. Greenwood 18/e, p 203]

16. **Beta phage is seen in:**
    a. Bacillus anthracis
    b. Corynebacterium diphtheriae
    c. Clostridium botulinum
    d. Peptostreptococci *[Ref. Jawetz, 27/e, p 193]*

Answers 1. **b.** Toxin... 2. **a.** Diphtheria 3. **a.** Loffelers 4. **a.** Mocarditis 5. **c.** Klebs-loeffler bacillus
6. **a.** Cell membrane 7. **a.** Susceptibility... 8. **b.** Isolation in... 9. **c.** Consists of... 10. **b.** Gram positive...
11. **d.** Loeffler's ... 12. **d.** C diphtheriae 13. **b.** Laryngeal 14. **c.** Toxigenic 15. **a.** Corynebacterium...
16. **b.** Corynebacterium diphtheriae

CHAPTER

12

Actinomycetes and Bacillus

ACTINOMYCETES

- Transitional forms between bacteria and fungi.
- Gram-positive, nonmotile, nonsporing, noncapsulated filaments.
- It includes:
  a. Actinomyces
  b. Nocardia

I

- Branching Gram positive bacilli.
- Grows best under anaerobic or micro-aerophilic conditions with the addition of 5–10% CO_2
- Actinomyces israelii is the most common cause of human actinomycosis.

ACTINOMYCES

- Anaerobic Gram-positive bacillus cause: Lumpy jaw (in cattle) and actinomycosis in human.

Actinomycosis

- **It is endogenous** infection since Actinomyces normally present in mouth, intestine, vagina.
- MC causative agent A. israelii.
- A. israelii is differentiated from other atinomyces species by gel diffusion and immunofluorescence.
- Usually a cooperative disease, i.e. Actinomycosis is usually accompanied by other associate bacteria which may enhance the pathogenic effect.
- Characterized by indurated swelling (mainly in connective tissue), suppuration, multiple sinuses towards skin with discharge of sulphur (black) granules.

Types of Actinomycosis

- **MC type cervicofacial**
  - **MC site** of cervicofacial is lower jaw (lumpy jaw) often adjacent to carious tooth.
- **Abdominal** – Usually around caecum.
- Thoracic
- **Pelvic** – In association with **IUCD's.**
- **Mycetoma**

I

Most common type of actinomycosis: Cervicofacial.
MC site of cervicofacial actinomycosis: Lower jaw

Mycetoma

- Painless localized woody induration without systemic symptoms. Granulomatous involvement of subcutaneous and deeper tissue induced by traumatic inoculation of saprophytic fungi or bacteria.
- ***MC* site foot,** present as tumor with multiple discharging sinus called as ***Madura foot.***
- ***MC* cause** is fungi called as eumycotic mycetoma/Maduramycosis/Madura foot. Black granules, stout filament seen on microscopy.
- Fungal agents of mycetoma: - *Pseudallescheria boydii* - *Exophiala jeanselmei*
  - *Madurella mycetomatis* - *Acremonium falciforme*
  - *Madurella grisea*
- Bacterial mycetoma are usually caused by *Actinomyces, Nocardia, Streptomyces, Nocardiopsis.*
- Even *S. aureus* and other pyogenic bacteria may cause mycetoma like lesion called **botryomycosis.**
- In actinomycotic mycetoma - Granules are white to yellow and thin filaments seen on microscopy.

Treatment • *Actinomycetoma:* Streptomycin + Dapsone or cotrimoxazole
• *Eumycetoma of Madurella mycetoma:* Keto / Itra-conazole
• *Other Eumycetoma:* rarely responds to chemotherapy

Diagnosis of Actinomycosis

- Specimen
  - Sputum, aspirations, biopsies
  - Shake it in test tube with saline - Sulphur granules can be seen by naked eyes in some case and by microscope in remaining.
- Microscopy
  - Granules are infact, bacterial colonies
  - Gram-positive filaments in the form of radiating ***club shaped = sun ray appearance*** seen.
  - Club is formed due to antigen-antibody reaction
  - Culture on solid media-shows spidery colonies which later develop into "molar tooth" colonies.

Note: Though sulfur granules are characteristic of actinomycosis, also they can be seen in mycetoma and botyromycosis.

Treatment

- **Penicillin is *drug of choice***

NOCARDIA

- **Aerobic, partially urease positive, catalase positive, partially acid fast,** causing exogenous infection, Nocardiosis (which refers to invasive disease).
- ***MC*** species associated with invasive disease **N. asterodes.**
- ***MC* risk factor** - Contact with soil or vegetable matter.
- Cell mediated immunity is important as neutrophil limit its growth but not kill them efficiently.
- Nocardiosis is usually initiated by inhalation and there is no person to person transmission. *... Jawetz 27/e, p 299*

Nocardia
- Gram positive, branched, strictly aerobic bacteria, that resemble rapidly growing mycobacteria.
- **MC cause of pulmonary nocardiosis:** N.asteroides.
- MC cause of cutaneous nocardiosis is N. brasiliensis.

Clinical Manifestation

- ***MC*** manifestation is **pneumonia** and disseminated disease which follows inhalation of bacteria.
- ***So, manifestation can be divided into:***
  a. *Respiratory tract disease* – ***MC* is pneumonia.** Prominent cough, small amount of thick purulent sputum that is odourless.
  b. *Extrapulmonary dissemination* – ***MC*** site **brain.** Typical manifestation is subacute abscess usually **supratentorial.** *... Harrison 19/e, p 1085, 18/e, p 1322*
  c. *Disease following transcutaneous inoculation* – Cellulitis, lymphocutaneous syndrome (most cases associated with *N. brasiliensis*), actinomycetoma (old fistula disappear with appearance of new fistula).

Diagnosis

- **First step:** Examine sputum or pus for crooked, branching, beaded, Gram-positive, acid fast (with weak acid) filaments, It also takes silver stains.
- In nocardial pneumonia sputum smears are often negative and diagnosis may require sampling through bronchoscopy or lung aspiration.
- Transtracheal aspiration should be avoided as it frequently leads to cellulitis in tissue around puncture wound.
- ***Culture:***
  - It use paraffin as carbon source *so paraffin baiting is used for isolation.*
  - Relatively slow to grow colony may take up to 2 weeks to appear.
  - ***Selective media*** (Colistin-nalidixic acid agar, modified Thayer-Martin Agar or buffered charcoal yeast agar) improves recovery from mixed flora *... Harrison 19/e, p 1086*

- *Nocardia grow relatively slowly; colonies may take upto 2 weeks to appear*
- *Selective growth is favored by inoculation at 45°C*
- *Species is identified by analysis of 16S r RNA gene sequences.*

Treatment

- *DOC* **Trimethoprim - Sulfamethoxazole** *... CMDT 2014, p 1410*
- **Best** alternative oral drug minocycline
- **Best** parenteral drug amikacin.

I

Actinomyces spp.	***Nocardia spp.***
Faculative anaerobes	Obligate aerobes
Growth at 35-37°C	Variable temperature
Oral commensals	Saprophytes
Non-acid fast	Acid fast

BACILLUS

- Genus consist of ***sporogenous aerobic*** Gram-positive bacilli.
- They are generally ***motile*** with peritrichous flagella ***except*** anthrax bacilli.
- Spores are heat resistant and constitute the most common contaminants of bacteriological culture media.
- Its important species are:
  1. Bacillus anthracis
  2. B. cereus.

BACILLUS ANTHRACIS

- ***First*** pathogenic bacteria to be observed under microscope.
- ***First*** bacterium used for preparation of attenuated vaccine.
- ***First*** communicable disease shown to be transmitted by inoculation of infected blood.
- ***First*** bacillus to be isolated in pure culture and shown to possess spores.

I

B.anthrax:
- Large non-motile, sporing bacillus that grows on all media forming colonies with ground glass surface appearance.
- Capsule and toxin are encoded by plasmid PXO_2 and PXO_1 respectively.

Morphology

- Nonmotile, capsulated sporing Gram-positive bacilli.
- Aerobic and facultative anaerobic
- Capsule is polypeptide in nature ***(exception since usually capsule is of lipopolysaccharide in nature).***
- Bacilli arranged in Bamboo stick/box car like appearance.
- Colonies of *B.anthracis* are round and have a *"cut glass"* appearance in transmitted light.
- On staining with polychrome methylene blue, it shows M'Fadyeans reaction; which represents capsular material.
- *Spores* – Used in biological warfare or **bioterrorism.** Formed in culture or soil but never in animal body. Not stained by ordinary methods. Not cause bulging of vegetative cells (in comparison of clostridia spores).

Culture

- **Selective** medium: **PLET** medium.
- On ***Agar plates*** – frosted glass appearance seen.
- On microscopy it has Medusa head appearance.
- On ***Gelatin stab*** – characteristic Inverted Fir tree appearance.
- ***Solid Media*** with penicillin – String of pearl reaction.
- **Cut glass appearance** – In transmitted light.

I

Mc-Fadyean's Reaction, Strings of pearls reaction: Differentiate between B. cereus and B. anthracis

Resistance

- Bacilli remain viable in bone marrow for a week and in skin for 2 weeks.
- Spores are destructed by oxidizing agents H_2O_2, 4% $KMnO_4$, formaldehyde (called as *Duckering*)

Virulence Factor

i. *Capsular poly - D-glutamic acid:*
   - Plasmid mediated, inhibit phagocytosis. Loss of plasmid cause loss of virulence (basis of live attenuated anthrax spore vaccine).

ii. *Toxin*: Plasmid coded, complex of *three fractions:*
- ***Factor I or edema factor (EF)*** – Activated only intracellularly increases CAMP.
- ***Factor II or protective antigen factor (PA)*** – It is the fraction that binds to receptors on target cell surface and provides attachment sites for EF and LF. *Antibody* to PA is protective because it blocks first step in toxin activity i.e. binding to target cells.
- Lethal factor (LF) or factor III - cause cell death.
- EF + PA = Edema toxin
- LF + PA = Lethal toxin

Disease

I
- Cutaneous anthrax is the commonest type of anthrax.
- It is characterized by malignant pustule.

- Cause anthrax which occurs primarily in herbivores ***(zoonosis).***
- ***Humans*** are ***more resistant*** to anthrax than herbivores.
- Transmitted by spores, by contact with infected animals or contaminated animal products, insects bites, ingestion or inhalation.
- ***Human anthrax is of 3 types:***
  a. Cutaneous anthrax (Hide Porter's disease): ***MC*** (95%);
     - Characterized by ***malignant pustule,*** i.e. central eschar surrounded by nonpitting edema.
     - Toxemia always present.
     - Smear of vesicle fluid is used to confirm diagnosis by culture and animal inoculation.
  b. Pulmonary/inhalational anthrax/*Wool Sorter's disease:*
     - Typically cause hemorrhagic mediastinitis. Characteristic X-ray finding is *symmetric mediastinal widening.*
  c. Gastrointestinal anthrax:
     - *Rare* form

Laboratory Diagnosis

- *Microscopy:*
  - Gram-positive bacilli with M'Fadyean's reaction - presumptive diagnosis made.
  - Immunofluorescent microscopy can confirm identification.
- If sample is putrid, ***Ascoli's thermoprecipitin (ring precipitin)*** test is done which demonstrate anthrax antigen in tissue extract.
- Test for ***antibody by immunoassays*** are useful in *confirming* diagnosis.

I

B. anthracis:	***B. cereus***
Non Motile	Motile
Capsulated:	Non capsulated
Susceptible to γ phage	Non susceptible

Treatment

- *DOC* Penicillin
- ***In Penicillin allergy*** – Ciprofloxacin, erythromycin, tetracycline or chloramphenicol.

BACILLUS CEREUS

- Resembles *B. anthrax except* that it is *motile (Swarming), non-capsulated, not susceptible to gamma bacteriophage, not show 'string of pearls' reaction.*
- Isolated from feces and other sources on **MYPA** (mannitol, egg yolk, phenol red polymyxin agar).
- It cause two type of food poisoning manifest as nausea, vomiting and abdominal cramps (seen in both emetic and diarrheal types).

Features	Diarrheal type	Emetic type
Incubation period	8-16 hr	1-5 hr
Food	Cooked meat and vegetables	Exclusively by rice
Enterotoxin	Resemble heat labile toxin of **E.coli** Either preformed or produced in intestine	Resemble heat stable toxin of **S. aureus** Already preformed in rice
Clinical features	Fever, vomiting rare	Diarrhea is not common
Serotype	2, 6, 8, 9, 10, 12	1, 3, 5

Remember:

- Presence of B. cereus in patient stool is not sufficient for diagnosis since it may be present in normal stool specimen; concentration of 10^5 or more bacteria per gram of stool is considered diagnostic. *... Jawetz 27/e, p 182*
- It also cause eye infections; localized and systemic infections (occur in patient with medical device or IV drug users).

Note: In 2001 there were may cases of anthrax in USA. At that time, B. anthrax was used as bioterrorism agent. Spores of bacillus in powdered form were packed in envelop and posted. After this experience CDC (Center for Disease Control) have prepared guidelines for identification of anthrax. Any large non-motile Gram (+)ve bacteria which is non-hemolytic and catalase positive is presumed to be anthrax bacilli for treatment purpose.

Multiple Choice Questions

BACCILUS

1. **An abattoir worker developed pustule which later progress to necrotic ulcer. Which of the following stain is useful emonstration of organism from smear made from pustule ?** [AI 07; AIIMS May 2012, 06]
   a. Polychromic methylene blue
   b. Chalkofluor white
   c. Geimsa
   d. Modified kinyon stain

2. **A man, after skinning a dead animal, developed a pustule on his hand. A smear prepared from the lesion showed the presence of gram-positive bacilli in long chains which were positive for McFadyean's reaction. The most likely aetiological agent is:** [AI 04]
   a. Clostridium tetani
   b. Listeria monocytogenes
   c. Bacillus anthracis
   d. Actinomyces sp

3. **A malignant pustule is a term used for:** [AIIMS 03]
   a. An infected malignant melanoma
   b. A carbuncle
   c. A rapidly spreading rodent ulcer
   d. Anthrax of skin

4. **Characteristic of Bacillus cereus food poisoning is:** [AIIMS Nov 10]
   a. Presence of fever
   b. Presence of abdominal pain
   c. Absence of vomiting
   d. Absence of diarrhoea

5. **Which of the following is true regarding anthrax:** [PGI 01]
   a. M'Fadyean reaction shows capsule
   b. Humans are usually resistant to infection
   c. Less than 100 spores can cause pulmonary infection
   d. Gram stain shows organism with bulging spores
   e. Sputum microscopy helps in diagnosis

6. **Noninvasive diarrhea can be caused by the following?** [AI 09]
   a. Shigella
   b. B. cereus
   c. Salmonella
   d. Y. enterocolitica

7. **A patient present with vomiting he had eaten rice 6 hour before. The most probable cause is:** [PGI 07]
   a. Bacillus cereus
   b. Staph. aureus
   c. Cl. difficle
   d. All

8. **Malignant pustule is caused by:** [AIIMS Nov. 10]
   a. B. Anthracis
   b. Leishmaniasia
   c. Basal cell carcinoma
   d. Pyoderma

9. **Malignant pustule is/are seen in infection with:** [PGI 11]
   a. Treponema pallidum
   b. Compylobacter granulomatosis
   c. Bacillus anthracis
   d. H. ducreyi
   e. Pseudomonas aeruginosa

10. **An abattoir worker presented with a malignant pustule on his hand that progressed to form an ulcer. Smear was taken from the ulcer and sent to laboratory for investigation. The diagnosis is:** [AIIMS Nov 2012]
    a. Cutaneous anthrax
    b. Carbuncle
    c. Ulcerating melanoma
    d. Infected rodent ulcer

ACTINOMYCOSIS

11. **Which of the following is the most predominant constituent of sulfur granules of Actinomycosis:** [AIIMS 04, 02]
    a. Organisms
    b. Neutrophils and monocytes
    c. Monocytes and lymphocytes
    d. Eosinophils

12. **A farmer present with multiple discharging sinuses in the leg not responding to antibiotics. Most likely diagnosis is:** [AIIMS 02]
    a. Madurella
    b. Actinomycetoma
    c. Nocardia
    d. Sporothrix

13. **Actinomycotic mycetoma is caused by:** [PGI 05]
    a. Actinomyces
    b. Nocardiasis
    c. Streptomyces
    d. Madura mycosis
    e. Staphylococcus

NOCARDIA

14. **Characteristic infection of Nocardia asteroides is:**
    a. Diarrhea[AI 2012]
    b. Secondary dissemination to liver
    c. Brain abscess
    d. Colonic diverticula

15. **The causative organism of Mycetoma is:** [PGI 02]
    a. Nocardia
    b. Dimorphic fungus
    c. Aspergillus
    d. Dermatophytes

16. **Nocardia is stained by:** [AIIMS 08]
    a. Acid fast stain
    b. Kiram's stain
    c. Alcian blue
    d. Mucin stain

17. **A clinical specimen was obtained from the wound of a patient diagnosed as nocardiosis. For the selective isolation of Nocardia sp. which one of the following would be the best method:** [AIIMS 04]
    a. Paraffin bait technique
    b. Castaneda's culture method
    c. Craige's culture method
    d. Hair bait technique

18. **Nocardia is differentiated from Actinomyces by:** [PGI 02]
    a. Gram stain
    b. ZN stain
    c. Nocardia causes mycetoma, Actinomyces do not
    d. Nocardia is faculative anaerobe

19. **True about Madura foot?** [PGI Dec 2008]
    a. Can erode bones
    b. Spread to lymph nodes
    c. Most commonly occur in hand
    d. Slow growing
    e. Antibiotic has no role

20. **A patient comes with history of unresponsive fever and cough. X-ray revealed pneumonia. Sputum examination showed gram positive, partially acid fast bacteria with branching filaments that grows on sheep blood agar. The most likely etiologic agents is:** [AI 2011]
    a. Actinomycetes
    b. Nocardia
    c. Aspergillosis
    d. Pneumococci

21. **A person 6 hr after consuming rice pudding in restaurant develops vomiting. True statements regarding food poisoning:** [AIIMS 2011]
    a. Caused by Staphylococcus aureus
    b. Preformed toxin
    c. Caused by vibrio parahaemolyticus
    d. Heat labile toxin
    e. Culture of food is more useful than stool

Explanations and References with Illustrative Answers

1. **Ans.(a) Polychromic methylene blue** *Ref. Ananthanarayan 8/e, p 245, 9/e, p 247*

It is case of cutaneous anthrax in abattoir worker (slaughter house worker).

- Cutaneous anthrax is also common in dock workers (who carry loads of hides and skin on their bare backs), butchers, farmers, veterinarians, workers involved in meat packing.
- Pulmonary anthrax is common in workers of wool factories.
- Intestinal anthrax occur in communities who eat carcasses of animals dying of anthrax.
- **Stains used in case of anthrax:**
  - Gram's stain
  - Sudan black B
  - Polychrome methylene blue (stains capsule = M'Fadyean reaction).

2. **Ans. (c) Bacillus anthracis** *Ref. Ananthanarayan 8/e, p 242, 245, 9/e, p 247; Harrison 17/e, p 1344, 18/e, p 1769*

This is typical presentation of cutaneous anthrax.

Bacillus Anthracis

- Gram-positive, aerobic, catalase positive, non-motile, capsulated **(polypeptide in nature),** spore forming bacilli.
- Spore are formed in culture or in the soil but never in the animal body during life and do not cause bulging of vegetative cell (In comparison of Clostridium spores).
- Spores are highly stable and can remain viable for decades. This remarkable stability makes them an ideal bio-weapon.
- Chain of bacilli have bamboo stick or box car like appearance.
- When blood film containing anthrax bacilli is stained with polychrome methylene blue and then examined under microscope, amorphous purplish material representing capsular material is noticed around bacilli. This is called **M'Fadyean's reaction (characteristic of anthrax bacilli)** and is used for presumptive diagnosis of anthrax in animals.

Cultural Characteristic

- Agar : **Frosted glass** appearances
- Microscopy : **Medusa head** appearance
- Gelatin stab : **Inverted fir tree** appearance
- **String of perals reaction** : **For differentiating B. anthrax from B. cereus and other aerobic spore bearers.**

Clinical Features

- Anthrax is zoonotic disease primarily of herbivores. Humans are more resistant than animals.
- Human become infected when spores are introduced into body by contact with infected animal or contaminated animal products, insect bites, ingestion, inhalation.

TYPES OF HUMAN ANTHRAX		
Cutaneous anthrax (Hide Porter's disease) (= Malignant pustule)	**Pulmonary anthrax (Wool Sorter's disease) (= Inhalation anthrax)**	**Gastrointestinal anthrax**
• **MC type** of anthrax	• *Earliest symptom are* typically viral like prodrome with fever, malaise, abdominal or chest symptoms	• Rare form
• Usual sites arms, hand, face, neck	• 100% fatal though with prompt treatment, survival is possible	• High mortality rate
• Characterized by presence of malignant pustule – Central necrotic painless lesion covered by black eschar surrounded by satellite lesions	• Characteristic X-ray mediastinal widening, hemorrhagic pleural effusion	• Primary lesion is most often located on tonsil
• Generally resolved spontaneously but 10-20% patients develop fatal septicemia		
• Also caused by shaving brushes of animal hair and occasionally by insect bite		

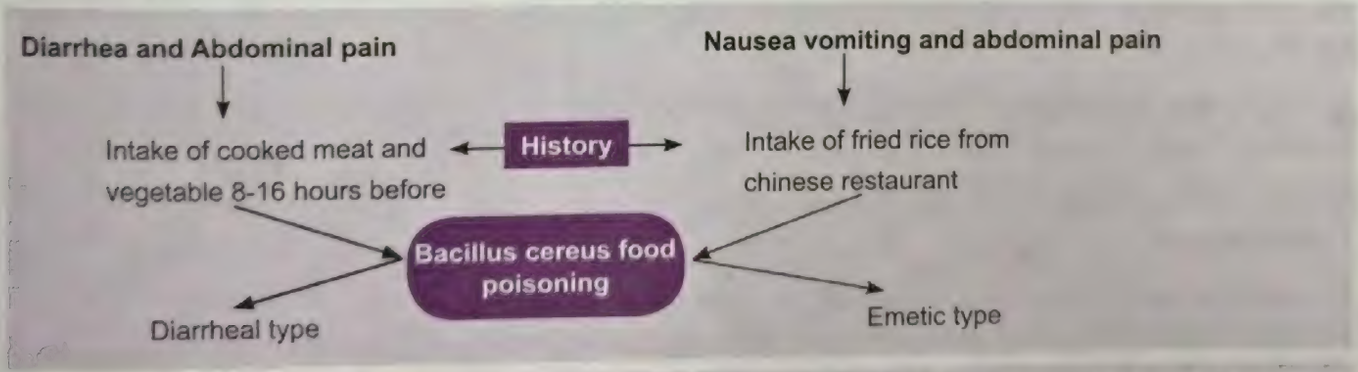
Remember:
- 10,000 spores are required to produce lethal disease in 50% of animals (LD_{50}). Though sometime few as minimum as one to three spore may be adequate to produce disease.
- Incubation period of cutaneous anthrax is 1 to 7 days while that of pulmonary anthrax may be as long as 6 weeks.

..... Jawetz 25/e, p 166

3. **Ans. (d) Anthrax of skin** *Ref. Ananthanarayan 8/e, 245, 9/e, p 247*
Cutaneous anthrax is also known as Hide Porter's disease or malignant pustule.

Remember: *Carbuncle is infection of 2 - 3 hair follicles*

4. **Ans. (b) Presence of abdominal pain** *Ref. Ananthanarayan 8/e, 247*
Abdominal pain is seen in both emetic and diarrheal type of B. cereus food poisoning.



Remember: A special media mannitol - egg yolk - phenol red-polymyxin agar (MYPA) medium is useful for isolating B. cereus from feces. Fever is common in invasive diarrhea and is rare in toxin mediated diarrhea.

5. **Ans. (a, b and e) M'Fadyean reaction shows capsule, Humans are usually resistant to infection and Sputum microscopy helps in diagnosis** *Ref. Harrison 15/e, p 914; Ananthanarayan 8/e, 246, 9/e, p 247-248*

Diagnosis of Anthrax

1. **Microscopy:**
   - Examination of cut piece of ear or swab soaked in blood of animals, it reveals gram-positive bacilli and positive M'Fadyean reaction; presumptive diagnosis is made.
   - Immunofluorescent microscopy confirms the diagnosis.

2. Any large gram-positive bacillus with morphology and cultural features of anthrax, i.e. non-motile, non-hemolytic on blood agar, catalase positive - presumptive report of anthrax can be given.

Diagnosis of Anthrax		
Initial confirmation	**Further confirmation**	**For epidemiological studies and strain characterization**
Lysis by gamma phage and direct fluorescent antibody test (DFA) for capsule specific staining and for polysaccharide cell wall antigen	By PCR for bacillus specific chromosomal markers	MLVA (multiple locus variable number tandem repeat analysis) and **AFLP** (amplified fragment length polymorphism) can be done

Remember: For Animal suspected to have died due to anthrax, autopsy is not permitted as the split blood can contaminate soil.

6. **Ans. (b) Bacillus cereus** *Ref. Harrison 17/e p 816, 18/e, 1088; Ananthanarayan 9/e, 249*

Diarrhea of *B. cereus* is mediated by enterotoxin which resemble *E. coli* LT. *The incubation period is 8-16 hours.*

Bacteria causing invasive diarrhea
- Shigella
- Salmonella
- Enteroinvasive E. coli
- Campylobacter jejuni

7. **Ans. (a) Bacillus cereus** *Ref. Ananthanarayan 8/e, 247, 9/e, p 249*

This is a characteristic presentation of B.cereus food poisoning (emetic type).

Bacillus cereus food poisoning
- *Produces two type of food poisoning:*
  a. *Emetic type* : It is associated with contaminated frid rice; the organism is common in uncooked rice, and its heat-resistant spores survive boiling. It is mediated by staphylococcal type of enterotoxin.
  b. *Diarrheal type :* Mediated by enterotoxin resembling E. coli. IP of diarrheal type is more in comparison to emetic type
- *Diagnosis:* The presence of B cereus in patient's stool is not sufficient to make a diagnosis of B cereus disease, since the bacteria may be seen in normal stool specimens; a concentration of 105 bacteria or more per gram of food is considered diagnostic.

8. **Ans. (a) B. anthracis** *Ref. Ananthanarayan 8/e, 245, 9/e, p 247*
Already explained

9. **Ans. (c) Bacillus Anthracis** *Ref. Ananthanarayan 8/e, 245, 9/e, p 247*
Already explained

10. **Ans. (a) Cutaneous anthracis** *Ref. Ananthanarayan 8/e, 242, 9/e, p 247*
Already explained

11. **Ans. (a) Organism** *Ref. Ananthanarayan 8/e, 393, 9/e, p 390-391*
Actinomycetes are true bacteria (possess cell wall, prokaryotic nuclei, etc.) bearing superficial resemblance to fungi (form mycelium or branching filaments).
- Actinomyces cause actinomycosis in human.
- MC cause is **A. israelii**
- MC type of actinomycosis – Cervicofacial (lower jaw).

Diagnosis of actinomycosis is made by:

1. *Demonstrating organism in the lesion by microscopy:*
   - Specimen: Pus/sputum containing sulphur granules.
   - Granules are crushed, gram-positive filaments seen as 'sun-ray appearance'.
   - These granules are infact bacterial colonies.
2. *Isolation in culture:*
   - In thioglycollate liquid media : A. israelli as **fluffy ball** at bottom of tube.
   - Solid media : **Spidery colonies** of A. israelii

12. **Ans. (a) Madurella** *Ref. Harrison 17/e, p 1266, 18/e, p 1325*

Actinomycetoma, usually responds to antibiotics.

Treatment of Mycetoma	
Actinomycetoma	Eumycetoma
Prolonged combination chemotherapy, e.g. with streptomycin and either dapsone or cotrimoxazole, cotrimoxazole + amikacin	Rarely responds to chemotherapy, some cases caused by Madurella mycetomatic respond to ketoconazole itraconazole, posaconazole

13. **Ans. (a, b and c) Actinomyces, Nocardiasis and Streptomyces** *Ref. Jawetz 27/e, p 673*

Mycetoma

- Localized chronic granulomatous involvement of the subcutaneous and deeper tissue.
- **Two types:**

Eumycotic mycetoma = Fungal (More common)	Actinomycotic mycetoma = Bacterial
– Madurella mycetomatis	– Actinomyces
– Pseudoallescheria boydii	– Nocardia
– Madurella grisea	– Streptomyces
– Acremonium falciforme	– Nocardiasis
– Exophiala jeanselmei	

Remember:

- Most common site of mycetoma - foot - (Called as Madura foot)
- Staphylococcus is causative agent of botryomycosis.

14. **Ans. (c) Brain abscess** *Ref. Harrison 18/e, p 1323, 19/e, p 1085*

- Subacute abscess is the typical extrapulmonary manifestation of nocardia, the most common site of dissemination is brain.
- Nocardial brain abscess are usually supratentorial, multiloculated, may be single or multiple. Brain abscess tend to burrow into ventricles and may extend out into subarachnoid space. Otherwise Meningitis is uncommon and is usually due to spread from nearby abscess.

Remember: Other common site of dissemination skin, kidney, bone, muscle.

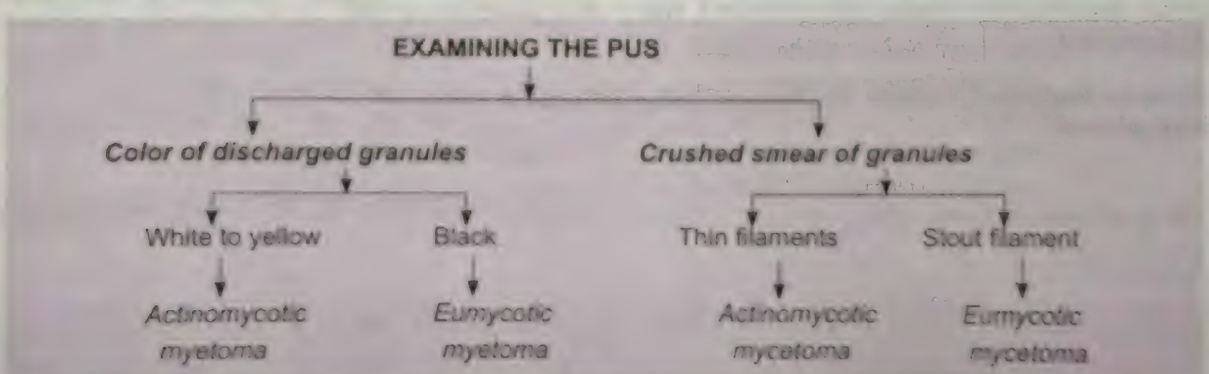
15. **Ans. (a) Nocardia** *Ref. Ananthanarayan 8/e, 393, 9/e, p 394; Jawetz 27/e, p 673*

Mycetoma

- Chronic granulomatous disease.
- Involve subcutaneous and deeper tissues destructing the contagious bone and fascia.
- **Site:** Foot (MC), hand, gluteal region and thigh often called as ***Madura foot.***
- It was first described from Madurai (South India).
- Presents as abscess, tumors with multiple sinuses discharging pus with sulphur granules.
- Granules are tightly clumped colonies of causative agent.

Diagnosed by:

A.



B. Isolation of agent by culture.

16. **Ans. (a) Acid fast stain** *Ref. Jawetz 27/e, p 199*

Nocardia is urease positive, catalase positive partially acid fast organism.

Stains for Nocardia

- **Acid fast staining** – Nocardia cell wall contains mycolic acid that are shorter chain than mycobacteria. If they are stained with routine acid fast reagant (carbol-fuchsin) and decolorized with 1-4% sulfuric acid instead of the stronger acid decolorant, most isolate will stain acid fast.
- **Silver stains.**

17. **Ans. (a) Paraffin bait technique** *Ref. Harrison 18/e, p 1324; Harrison 19/e 1086; Jawetz 27/e p 199*

Diagnosis of Nocardiosis

- **Specimen** – Sputum or pus or spinal fluid or urine or biopsy material. In case of pneumonia, sampling done by bronchoscopy or lung aspiration. Transtracheal aspiration should, however, be avoided as it frequently leads to nocardial cellulitis around the puncture wound.
  i. Microscopy – Crooked, branching, beaded, gram-positive filament seen.
  ii. Stain – They are **Acid fast** and also take silver stains.
  iii. Isolation – Paraffin baiting mixed culture's done as it use paraffin as carbon source
  – Selective media are Thayer Martin agar, Colistin-nalidixic acid buffered charcoal yeast extract agar.
- If brain involved – CT or MRI
- In actinomycetoma – granules are examined.

Remember:
- Castaneda culture is method of blood culture (e.g. S. typhi, Brucella).
- Craigie's tube is used for the separation of motile from non-motile bacteria and also used to obtain phase variants in Salmonella species.
- Recently Molecular methods such as—t RNA sequencing and RFLP analysis are approved for identification.

18. **Ans. (b) ZN stain** *Ref. Ananthanarayan 8/e, 392, 9/e, p 392-393; Harrison 17/e, p 994; Harrison 19/e 1084; Greenwood 18/e 235*

Both are gram-positive filaments causing mycetoma.

Features	Actinomyces	Nocardia
Morphology	Nonacid fast	Acid fast (Ziehl and Neelsen stain); Fite Faraco Method
Growth in media	Anaerobes	Aerobic
Mode of infection	Endogenous	Exogenous
Diseases	**MC** is cervicofacial actinomycosis (Lumpy Jaw)	**MC** is pneumonia and disseminated disease
Treatment	Penicillin G	Sulfonamides
Paraffin	Can't use	Can use

19. **Ans. (a) and (d) Can erode bones and Slow growing** *Ref. Ananthanarayan 8/e 393, 9/e, p 394; Harrison 17/e p 1265, 1266*
Already explained

20. **Ans. (b) i.e. Nocardia** *Ref. Harrison 17/e, p 994-995, 18/e, p 1323; Jawetz 27/e, p 199*
Already Explained

21. **Ans. (b) (e) i.e. Preformed, Culture** *Ref. Harrison 18/e, p 1088; Jawetz 27/e, p 199*
Already Explained

Chapter Review

1. **Most common cause of actino mycetoma in India:** [DNB 2013]
   a. Nocardia braziliensis
   b. Actinomadura madure
   c. Piedra
   d. Tinea crurin

   [Ref. Jawetz 24/e, p 220]

   - MC cause of mycetoma is fungi.
   - MC cause of Actinomycetoma are:
     — Nocardia brasiliensis
     — Streptomyces somaelinsis and
     — Actinomadura madurae

2. **All are causes of madura mycosis except:** [UP 02]
   a. *Streptomyces* b. *Nocardia*
   c. *Actino boydii* d. *Circumvento-geniculta*

   [Ref. Ananthanarayan 8/e, 393, 9/e, p 394]

3. **All are true about cutaneous anthrax except:** [UP 02]
   a. Extremely painful lesions
   b. The whole area is congested and edematous
   c. Central crustation with black eschar
   d. Satellite nodule around inguinal region

   [Ref. Ananthanarayan 8/e, 245, 9/e, p 247]

4. **A wool cutter is suffering from fever with a cervical lymph node enlargement for last 15 days, most likely he is suffereing from:** [UP 04]
   a. Anthrax b. Mycetoma
   c. Sporotrichosis d. Coccidimycosis

   [Ref. Ananthanarayan 8/e, 245,9/e, p 247]

5. **Actinomycosis caused by:** [Bihar 05]
   a. Gram+ve organism
   b. Gram–ve bacteria
   c. Anaerobic bacteria
   d. Fungus

   [Ref. Ananthanarayan 8/e, 392, 9/e, p 394]

6. **True regarding anthrax is all except:** [AIIMS 97]
   a. Caused by insect bite
   b. Caused by rubbing of skin
   c. Cutaneous type is rare nowadays
   d. Pulmonary infection occurs by inhalation

   [Ref. Ananthanarayan 8/e, p 245, 9/e, p 247]

   Cutaneous —anthrax in MC type of Anthrax

7. **Sulphur granules are seen in:** [UP 06]
   a. *Rhinoscleroma* b. *Actinomycetes*
   c. *Candida fungi* d. *Listeria-monocytogenes*

   [Ref. Ananthanarayan 8/e, 390, 9/e, p 391]

8. **Anthrax bacilli differes from anthracoid bacilli by being:** [Kar 06]
   a. Non-capsulated
   b. Strict aerobe
   c. Non-motile
   d. Hameolytic colonies on blood

   [Ref. Ananthanarayan 8/e, 247, 9/e, p 249]

   Differentiating features between anthrax and anthracoid bacilli

Anthrax Bacilli	Anthracoid Bacilli
– Nonmotile	– Generally motile
– Capsulated	– Noncapsulated
– Grow in long chains	– Grow in short chains
– Medusa head colony	– Not present
– No growth in penicillin agar (10 units/ml)	– Grow usually
– Hemolysis absent or weak	– Usually well marked
– Inverted fir tree growth and slow gelatin liquefaction	– Rapid liquefaction
– No turbidity in broth	– Turbidity usual
– Salicin fermentation negative	– Usually positive
– No growth at 45 °C	– Grows usually
– Growth inhibited by chloral hydrate	– Not inhibited
– Susceptible to gamma phage	– Not susceptible
– Pathogenic to laboratory animals	– Not pathogenic

9. **Which of the following is not true of Actinomycosis:** [AI 95]
   a. Demonstration of filaments
   b. Caused by *Actinomyces israelii*
   c. Organism cannot be cultured
   d. Sulphur granules in pus

   [Ref. Ananthanarayan 8/e, p 393, 9/e, p 393]

Answers 1. a. Nocardia... 2. d. Circumvento... 3. a. Extremely... 4. a. Anthrax 5. a. Gram+ve...
6. c. Cutaneous... 7. b. *Actinomy*... 8. c. Non-motile 9. c. Organism...

10. All of the following are true about anthrax except:
a. Plasmid is responsible for toxin prodcution **[AI 98]**
b. Cutaneous anthrax generally resolve sponta-neously
c. Capsular polypeptide aids virulence by inhibiting phagocytosis
d. Toxin is a complex of two fractions
[Ref. Ananthanarayan 8/e, p 244, 9/e, p 246]

11. Which of the following is false about mycetoma:
a. Can affect lower and upper extremities **[AI 96]**
b. Caused by actinomycetes and filamentous fungi
c. Diagnosis is by examination of pus
d. Uncommon in India
[Ref. Ananthanarayan 8/e, p 393, 9/e, p 394]

12. A discharging sinuses seen in: **[AI 95]**
a. Sporotrichosis
b. Cryptococcosis
c. Histoplasmosis
d. Mycetoma
[Ref. Ananthanarayan 8/e, p 393, 9/e, p 394]

Answers **10.** d. Toxin... **11.** d. Uncommon... **12.** d. Mycetoma

NEET Pattern Questions

1. **Bacillus anthracis toxin rediates its action through all except:**
   a. cAMP
   b. Stimulating macrophages
   c. TNFα
   d. cGMP *[Ref. Greenwood, 18/e, p 238]*

2. **A person developed severe vomiting after eating food from chinese restaurant, 3 hours before. Most likely causative agent is:**
   a. Staph. aureus
   b. B. cereus
   c. C. difficle
   d. Pseudomonas
   [Ref. Ananthanarayan, 9/e, p 249]

3. **Color of granule of actinomycetes:**
   a. Black b. Yellow
   c. Red d. Brown
   [Ref. Ananthanarayan, 9/e, p 392; Harrison 18/e, p1329]

4. **Actinomycosis most common site:**
   a. Cervicofacial b. Thorax
   c. Abdomen d. Brain
   [Ref. Ananthanarayan, 9/e, p392; Harrison 18/e, p1326]

5. **A patient is suffering from pneumonia. Laboratory study shows acid-fast filamentous bacterium. The causative organism is:**
   a. M. tuberculosis
   b. Actinomyces
   c. Nocardia
   d. Mycobacterium Avium intracellulare
   [Ref. Ananthanarayan, 9/e, p 393]

6. **Mc fadyean reaction seen with which organism:**
   a. Clostridium perfringens
   b. Clostridium botalinum
   c. Bacillus cereus
   d. Bacillus anthracis *[Ref. Ananthanarayan, 9/e, p 248]*

   Demonstration of capsular material through staining with polychrome methylene blue is called Mc Fadyean's reaction

7. **Medusa head colonies on nutrient agar is seen in:**
   a. Pneumococcus
   b. Legionella
   c. Brucella
   d. Anthrax *[Ref. Ananthanarayan, 9/e, p 245]*

8. **True about anthrax toxin are all except:**
   a. Has three fractions
   b. Increase cAMP
   c. Coded by plasmid
   d. Inhibits protein synthesis
   [Ref. Ananthanarayan, 9/e, p 246]

   Anthrax toxin acts by increasing cAMP

9. **Capsule of Bacillus anthracis is formed of:**
   a. Polysaccharide
   b. Lipopolysaccharide
   c. Polypeptide
   d. Long chain fatty acids
   [Ref. Ananthanarayan, 9/e, p 245]

10. **Actinomyces differs from bacteria in that:**
    a. Gram positive
    b. Filamentous organism
    c. Non-motile
    d. Non-acid fast *[Ref. Ananthanarayan, 9/e, p 391]*

11. **A child is presenting with vomiting and abdominal pain after 5 hours of eating some food. The most likely causative orgonism:**
    a. Bacillus cereus
    b. Cl. perfringens
    c. Cl. botulinum
    d. V. cholerae *[Ref. Ananthanarayan, 9/e, p 249]*

    This is emetic type of food poisoning of B. cereus

12. **A patient with sulphur granules discharging from sinus, suggestive of infection with:**
    a. Staphylococcus
    b. H ducryei
    c. Mycetoma
    d. Sporotrichosis *[Ref. Ananthanarayan, 9/e, p 391, p 601]*

Answers 1. d. cGMP 2. b. B. cereus 3. a. Black 4. a. Cervicofacial
5. c. Nocardia 6. d. Bacillus anthracis 7. d. Anthrax 8. d. Inhibits protein synthesis
9. c. Polypeptide 10. b. Filamantous... 11. a. Bacillus cereus 12. c. Mycetoma

CHAPTER

13 Listeria Monocytogenes

- Catalase positive, Gram positive non-spore forming coccoid rod (coccobacilli) with tendency to occur in chains.
- Shows slow tumbling motility at 20°-25°C and is non-motile at 37°C.
- Humans are accidental host.
- Monocytogenes is of interest not only to clinicians but also to scientist as a model intracellular pathogen that can be used to study basic mechanism of microbial pathogenesis and host immunity.

Culture

- Grows on ordinary media. (*Mueller-hinton agar*).
- Growth is better in blood agar or if material is stored in tryptose phosphate or thioglycollate broth at 4°C called *cold enrichment*.
- Growth is improved when cultures are incubated at reduced oxygen tension and with 5-10% CO_2
- *Listeria monocytogenes can be differentiated from other Listeria by:*
  - β hemolysis on sheep blood agar.
  - Production of acid from glucose and mannose but not from D.xylose.
- *Listeria monocytogenes* is divided into serotypes on the basis of somatic [O] or flagellar [H] antigen.
- Most human infections are caused by 1/2a, 1/2b, 4b.
- Human disease due to L.monocytogenes generally occurs in pregnancy or immuno-suppression.

I

- Catalase (+)ve
- Gram (+)ve
- Shows tumbling motility at 20-25°C and is nonmotile at 37°C
- Growth is better on cold enrichment

Mode of Transmission

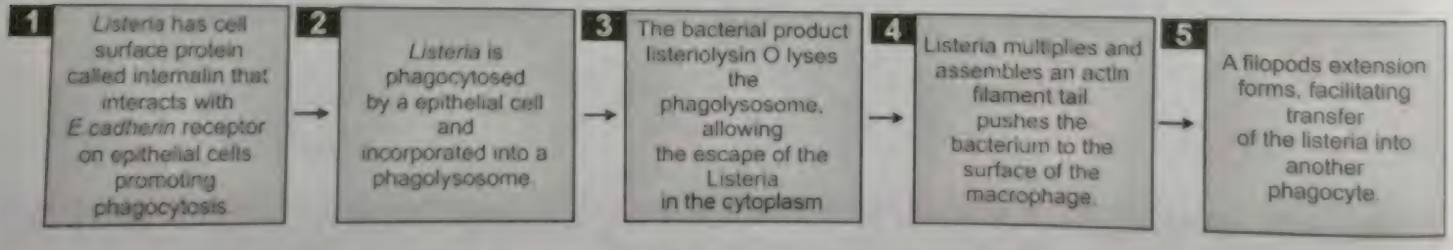
Food borne [Ready to eat food are most likely].
Human to human transmission is not seen (except vertical transmission from mother to fetus.)

Pathogenesis

- Intracellular pathogen [so, no role of humoral immunity] hence immunity is primarily cell - mediated.
- Lack of gastric acidity increase risk.
- Most important determinant of pathogenesis is its β hemolysin listerolysin O.

... Harrison 19/e, p 982, 18/e, p 1194

- **Life-cycle of *Listeria monocytogenes* in host macrophages includes following step.**



- Though the name *L. monocytogenes* is suggestive of monocytosis, monocytosis is not a hallmark of human infection, it is seen only in rabbits.

I

Listeria:
- Human listeriosis is almost always caused by L. monocytogenes.
- Non motile at temp ≥ 37°C and exhibit characteristic tumbling motility at 25°C
- Listeria is an intracellular bacteria
- Most infection are food borne.
- Fetal infection is acquired from mother

Clinical Presentation

1. *Pregnancy Associated Listeriosis*
   - Most infections detected in 3rd trimester.
   - Woman experiences mild illness characterized by fever, myalgia, backache.
   - Transplacental spread results in chorioamnionitis, premature labor, intrauterine fetal death, stillbirth, early onset disease of newborn, recurrent spontaneous abortion.
2. *Neonatal Listeriosis*

   A. *Early onset disease:*
   - Occur with in 7 days of birth. Most infants are symptomatic by 2nd day.
   - Transmitted by aspiration of infected amniotic fluid.
   - Present as:
     a. Intrauterine sepsis
     b. Respiratory distress
     c. Skin lesions
     d. ***Granulomatosis infantisepticum:*** – Characterized by abscesses involving liver, spleen, adrenal gland and other sites.
   - Mostly follow complicated labor.

   B. *Late onset disease:*
   - Occur between 7-21 days.
   - Mostly present as meningitis
   - Born at term by uncomplicated labor
   - Transmitted during passage through birth canal.
3. ***Listeriosis not associated with pregnancy***
   - ***MC*** underlying conditions are *chronic glucocorticoid therapy,* diabetes, solid and hematologic *malignancy (particularly fludarabine treated), liver disease, AIDS*
   - Bacteremic infection without evident focus is ***MC*** clinical manifestation while infection in CNS ranks **2nd** in which meningitis is ***MC.*** It can directly invade brain parenchyma producing ***cerebritis or focal abscess.***
   - *L.monocytogenes* is ***MC*** cause of *meningitis* in kidney ***transplant*** patient after ***1 month.***
   - *Listeria* meningitis must be considered in chronically ill patient with aseptic meningitis particularly when presentation is subacute. It is also related to 5-10 % of community acquired meningitis.
   - **Gastroenteritis**
     - Can be seen in immunocompetent individual within 48h of ingestion of contaminated food such as milk meat and salad.
     - Manifestation includes fever, diarrhea, headache.
   - It is also associated with basilar meningitis of cattles and sheep.

Diagnosis

- Invasive listeriosis is diagnosed when organism is cultured from blood, CSF or amniotic fluid.
- *Listeria* may be confused with diptheroids or pneumococci in Gram stained CSF or may be Gram variable and confused with *Hemophilus* sp.
- Antibody to listeriolysin O:
  - For epidemiological purpose.
  - For diagnosis of culture negative CNS infection.
- **Antons test** – Instillation into rabbit eye cause conjunctivitis.

Treatment

- IV administration of ***Ampicillin (DOC)*** or penicillin often in combination with aminoglycoside.
- ***Cotrimoxazole*** in case of pencillin allergy.
- Cephalosporins are not effective.

Multiple Choice Questions

1. **A 30-year-old woman with a bad obstetric history presents with fever. The blood culture from the patient grows Gram-positive small to medium coccobacilli that are pleomorphic, occurring in short chains. Direct wet mount from the culture shows tumbling motility. The most likely organism is:**
   a Listeria monocytogenes [AI 04]
   b. Corynebacterium sp.
   c. Enterococcus sp
   d. Erysipelothrix rhusiopathiae

2. **All the following are true about Listeria except:**
   a Transmitted by contaminated milk [AI 04]
   b. Gram (-)ve bacteria
   c. Causes abortion in pregnancy
   d. Causes meningitis in neonates

3. **A 3-week-old child presented to the pediatrician with meningitis. A presumptive diagnosis of late onset of perinatal infection was made. The CSF culture was positive for gram-positive bacilli which of the following characteristics of this bacteria would be helpful in differentiating it from other bacterial agents?**
   a Ability to grow on blood agar [AIIMS 05]
   b. Ability to produce catalase
   c. Fermentative attack on sugars
   d. Motility at 25°C

4. **A major step in the pathogenesis of listeriosis is:**
   a. The formation of antigen-antibody complex with resultant complement activation and tissue damage
   b. The release of hyaluronidase by L. monocyto-genes, which contributes to its dissemination from local sites [AIIMS 05]
   c. The antiphagocytic activity of the L. monocyto-genes capsule
   d. The survival and multiplication of L. mono-cytogenes within mononuclear phagocytes and host epithelial cells

5. **In patient with Listeria meningitidis who is allergic penicillin the treatment of choice is:** [AIIMS 04]
   a Vancomycin
   b. Gentamycin
   c. Trimethoprim - sulfamethoxazole
   d. Ceftriaxone

6. **Which of the following is not true regarding transmission?** [AI 09]
   a. Legionella – Through water aerosols
   b. Listeria – Refrigerated food
   c. Leptospirosis – Rat urine
   d. Tetanus – Droplets/Dust

7. **Listeria culture media:** [AIIMS May 09]
   a. Baker
   b. Korthoff
   c. Tinsdale
   d. Blood agar

8. **A 28 year old lady presented with, headache, kernigs sign positive, culture showed gram positive bacilli, most probable organism is:** [AI 2011]
   a. Listeria monocytogenes
   b. H. influenza
   c. Meningococci
   d. Streptococcus pneumoniae

9. **Gram (+)ve bacilli causing meningitis:**
   a. Pneumococci [AIIMS Nov 2014]
   b. Listeria
   c. E. coli
   d. Meningococci

Explanations and References with Illustrative Answers

1. **Ans. (a)** ***Listeria monocytogenes*** *Ref. Ananthanarayan 8/e, p 394, 9/e, p 395*

Tumbling motility is characteristic of Listeria monocytogenes - *(other three are non-motile).*

Listeria monocytogenes

- Catalase positive, non-sporing-gram positive, Cocco bacilli.
- Tendency to occur in chains.
- Characteristicly **show tumbling motility at 25°C and at 37°C** is non-motile because Peritrichous flagella are produced optimally at 20 to 30°C but only scantily or not at all at 37°C.
- Grows best between 30°C and 37°C (temperature range is 1 to 45°C).
- Aerobic or microaerophilic.
- Intracellular as well as able for direct cell to cell spread so not eliminate by antibodies and cause infection in deficient cell mediated immunity.

Lab diagnosis of Listeria Monocytogenes

- **Specimen:** Blood, pus, CSF, swab from cervical and vaginal secretion, cord blood.
- **Microscopy:** Usually negative. In rare case extra and intracellular coccobacilli may be seen.
- **Culture:** – Listeria can grow on ordinary media, but growth is better on blood agar or tryptose phosphate agar. On blood agar, Listeria form small colonies surrounded by a narrow zone of β hemolysis. The bacilli are actively motile (tumbling motility) when grown at 25°C. Biochemical tests are required to differentiate L. monocytogenes from other Listeria species.
  – Growth is improved when cultures are incubated at reduced oxygen tension.
- **Biochemical test:** L. monocytogenes is always D.xylose negative and d-methyl-D-mannoside positive.
- **Rapid detection:** – Selective enrichment broths based on immunoassays are commercially available for rapid detection of Listeria from food species.
- **Serology:** – Serological test and PCR assays are not useful in diagnosing clinical infection at present.

Remember:
- E. rhusiopalthae is α-hemolytic non-motile Gram-positive bacillus with tendency to form long filaments.
- Its **MC** infection in humans is called erysipeloid = Seal finger = whole finger.

2. **Ans. (b) Gram –ve bacteria** *Ref. Harrison 19/e, p 982 - 983, 18/e, p 1195*

Already explained, please See Ans. 1

3. **Ans. (d) Motility at 25° C** *Ref. Ananthanarayan 8/e, p 395, 9/e, p 395*

- This is a case of **'Late onset neonatal meningitis'** of Listeria monocytogenes as culture reveals Gram-positive bacillus.
- Bacterial cause of neonatal meningitis are:
  – E. coli > Group b streptococci (Strep. agalactiae) > other gram-negative bacilli > L. monocytogenes.

..... Forfar and Anelus textbook of pedia 319, 1338

- E. coli is Gram-negative bacilli while group b streptococci is gram-positive cocci.

Important bacterias causing meningitis

Features	Listeria	E.coli	Streptococci	Staphylococci	H. influenzae
Ability to grow on blood agar	+	+	+	+	+
Production of catalase	+	+	–	+	+
Fermentation of sugars	+	Both acid and gas are produced	+	+	+
Motility at 25°C	+	–	–	+	–

Remember:
- Catalase production and β hemolysis is used to differentiate Listeria monocytogenes from other Listeria not from other bacterias.
- Only Listeria and E.coli are motile in above mentioned bacterias.

4. **Ans. (d) The survival and multiplication of *L. monocytogenes* within mononuclear phagocytes and host epithelial cells**
Ref. Jawetz 27/e, p 196 - 198

Pathogenesis of Listeria Monocytogenes

Listeria
↓
Enters body through GIT after eating contaminated foods
↓
Interlin interacts with E. cadherin (recepter on epithelial cells)
↓
Phagocytosis of *Listeria* in epithelial cells forming phagolysosome
↓
In phagolysosome bacteria produce lysteriolysin which lyse the membrane of phagolysosome
↓
Listeria escapes into cytoplasm
↓
Induce host cell actin polymerization
↓
Listeria is propelled to cell membrane
↓
In membrane *Listeria* forms protrusion called filopods
↓
Filopods is ingested by nearby epithelial cells or macrophages
↓
Listeria is released and cycle begins again

So, in this way Listeria can move from cell to cell without being exposed to antibodies, complement or polymorphs.

Remember:
- Iron is important virulence factor of Listeria
- Shigella flexneri and rickettsia also use the host cell actin and contractile system to spread infection.

5. **Ans. (c) Trimethoprim - sulfamethoxazole** *Ref. Harrison 19/e, p 983, 18/e, p 1196*
   - DOC for listeriosis (non-pregnant, neonate, pregnant) is ampicillin or penicillin often in combination with aminoglycosides.
   - During last month of pregnancy (in case of penicillin allergy), may be treated with erythromycin.
   - Otherwise in all cases of penicillin allergy **DOC** is trimethoprim-sulfamethoxazole.

6. **Ans. (b) Listeria: Refrigerated food** *Ref. Harrison 18/e, p 1195; 19/e p 982; Chakoraborty 2/e, p 453*

Mode of transmission of listeriosis
   - Food borne by ready to eat food, milk, deli meat, salad.
   - Through birth canal at the time of delivery
   - Nosocomial through contaminated resuscitation equipments of newborn.

7. **Ans. (d) Blood agar** *Ref. Chakraborty 2/e, p 454; Ananthanarayan 8/e, p 395, 9/e, p 395*
Already explained, in Ans. 1

8. **Ans. (a) i.e. Listeria monocytogenes** *Ref. Ananthanarayan 8/e, p 395, 9/e, p 395; Harrison 17/e, p 2621, 19/e, p 982*
Among options provided listeria is the only Gr positive bacilli. Listeria accounts for 10% of cases of community acquired bacterial meningitis.
For details see previous answers.

9. **Ans. (b) i.e. Listeria** *Ref. Harrison 18/e, p 3410*
Already explained

NEET Pattern Questions

1. **True statement about listeria:**
   a. Gram negative bacillus
   b. Motile by peritrichous flagella
   c. Commonest cause of community acquired meningitis
   d. Only one serovar is known
   [Ref. Ananthanarayan, 9/e, p 395]

2. **Anton test is used for:**
   a. Listeria monocytogenes
   b. Ligaonella
   c. Brucella
   d. Bordetella *[Ref. Ananthanarayan, 9/e, p 395]*

Anton's test: Instillation of culture containing listeria into the eye of rabbit or guinea pig produces severe kerato conjunctivitis

3. **Most common serotype of listeria causing infections:**
   a. 1/2a b. 1/3a
   c. 1/3b d. 1/2c
   [Ref. Ananthanarayan, 9/e, p 395]

4. **Tumbling motility is shows by:**
   a. Listeria monocytogenes
   b. Proteus vulgaris
   c. Borrelia
   d. Clostridla *[Ref. Ananthanarayan, 9/e, p 395]*

5. **Mode of transmission of listeria:**
   a. Ingestion
   b. Inhalation
   c. Skin inoculation
   d. None *[Ref. Harrison, 19/e, p 982]*

6. **All are true about listeria except:**
   a. Gram positive
   b. PALCAM agar is used for isolation
   c. Characteristic tumbling motility at 37°C
   d. Umbrella shaped growth
   [Ref. Ananthanarayan, 9/e, p 395]

7. **Listeria resists phagocytosis in phagosomes (phagolysosomes) due to:**
   a. β-hemolysin
   b. Caspases
   c. Cell membrane adhesion molecules
   d. Opacity associated protein (OAP)
   [Ref. Harrison, 19/e, p 982]

Listeriolysin O, a β-hemolysin is largely responsible for mediating the rupture of phagosomal membrane that forms after phagocytosis of L. monocytogenes.

8. **Granulomatous infantisepticum is caused by:**
   a. HSV-1
   b. HSV-2
   c. Listeria
   d. Group B streptococcus *[Ref. Harrison, 19/e, p 983]*

Answers 1. **b.** Motile by peritrichous flagella 2. **a.** Listeria monocytogenes 3. **a.** 1/2a
4. **a.** Listeria monocytogenes 5. **a.** Ingestion 6. **c.** Characteristic
7. **a.** β-hemolysin 8. **c.** Listeria

CHAPTER

14

Mycobacteria

Gram-positive, **Aerobic,** acid fast, non-motile, noncapsulated and non sporing fungus like bacteria.

Classification of mycobacteria is as follows:

1. **Tubercle bacilli -** *M. tuberculosis, M. bovis, M. africanum*
2. **Lepra bacilli -** *M. leprae*
3. **Mycobacteria causing skin ulcers -** *M. ulcerans, M. haemophilum, M. marinum* or balnei
4. **Atypical mycobacteria** = Nontuberculous = Paratubercle = MOTT (Mycobacteria other than Tuberculosis)

Classification of Atypical Mycobacteria	
Group I	Photochromogenes e.g. M. kansasii, M. marinum, M. simiae, M. asiaticum
Group II	Scotochromogens e.g. M. scrofulaceum, M. gordonae, M. szulgai, M. flavescens.
Group III	Nonphotochromogens e.g. M. avium, M. intracellulare, M. xenopi, M. ulcerans, M. malmoense, M. celatum, M. hemophilum, M. gastri, M. genavense, M. shimoidei, M. trivale, M. terrae, M. nonchromogenicum.
Group IV	Rapid growers – M. fortuitum, M. chelonae Chromogenic rapid growers are saprophytes e.g. M. smegmatic, M. phlei.

5. Johne's bacillus - *M. paratuberculosis*

I

Gram positive acid fast
Non-motile
Non-capsulated

Mycobacteria Leprae (Hansen bacillus)

Morphology

- Obligate intracellular bacilli with polar bodies and intracellular elements, resist decolorization with 5% H_2SO_4 (acid fast).
- Live bacilli in tissue appear solid and uniformly stained.
- *Morphological index (MI)* is a measure of number of viable *M. leprae.*
- Dead bacilli - fragmented and granular appearance.
- *Bacteriological index (BI)* - number of *bacilli* in tissue, include both live and dead.
- Increasing BI and MI suggests relapse/drug resistance.
- Bacilli can be seen as single or multiple in large undifferentiated histiocytes (as *M. leprae* is intracellular bacilli).
- Commonly multiple bacterias are seen in the cells (called as Virchow's lepra cells or foamy cells) as agglomerates. Bacterias are bound by lipid like substances to form globi in cells. In globi they are arranged in parallel rows which resembles ends of cigarettes in cigar bundle.
- Strain variability is demonstrated recently.
- *(PGL-I) phenolic glycolipid act as virulence factor.*

I

M.leprae:

- Gram positive, acid fast, obligate intracellular parasite.
- Phenolic glycolipid acts as a virulence factor.
- O-diphenoloxidise is an enzyme characteristic of leprosy bacilli.
- **Generation time** - 12-13 days

Culture

- Unique in exhibiting dopa oxidase activity and acid fastness that is pyridine extractable.
- *Not grow in artificial media* but multiply in foot pad of mice at low temperature of 20°C.
- Nine banded *armadillo* (*Dasypus novemcinctus*) is highly *susceptible to* it.
- Grows **best in** cooler tissues (skin, peripheral nerves, anterior chamber of eye, upper respiratory tract, testis) **sparing** warmer areas (axilla, groin, scalp, midline of back, ovary).

I

- Principal target is Schwann cells.
- First sign of leprosy is a non-specific indeterminate skin lesion which often heal spontaneously.

I

M. leprae cannot be cultivated in vitro due to loss or disruption of many genes in artificial media

Transmission

- Nasal droplet, contact with infected soil, insect vectors. Skin to skin contact is not an important route. Physicians and nurses caring for leprosy patient are not at risk.

Clinical Features

- It causes Leprosy (Hansen's disease) having spectrum of manifestations.
- Incubation period generally varies from 5-7 years.
- Two extremes or polar form of disease are the lepromatous or tuberculoid types.

Note: Risk of leprosy is not increased in AIDS patient.

Tuberculoid Leprosy

- Symptoms confined to skin and peripheral nerves.
- **MC nerve involved -** Ulnar, posterior auricular, peroneal and posterior tibial nerve.
- Invasion and destruction of nerves in dermis by T1 cells (by bacilli in LL) are **pathognomic for** leprosy.
- Medial popliteal **nerve** never involved.

Lepromatous Leprosy:

- Bacilli are present in blood and in all organ system **except lungs and CNS**. Even than patient are afebrile and not susceptible to opportunistic infection.
- Leprosy bacilli are numerous in the skin where they are often found in large clumps (***globi***) and in peripheral nerves where they initially invades Schwann cells.

Other important clinical features are as follows:

Clinical & Histologic features	Tuberculoid (TT) Borderline	Borderline >Tuberculoid (BT) Leprosy	Mild-Borderline (BB) Leprosy	Borderline Lepromatous (BL)	Lepromatous Leprosy (LL)
	MC type in India		**Most unstable leprosy**		
Skin lesions	Up to 3 in number; sharpy defined, hypopigmented asymmetric macules or plaques with tendency toward central clearing, elevated borders	Smaller or larger than in TT; potentially more numerous than in TT; usually annular lesions with sharp margination on exterior & interior borders; borders not as elevated as in TT	Dimorphic lesions intermediate between BT & BL	LL-type lesions; ill-defined plaques with an occasional sharp margin; few or many in number, shiny appearance	Symmetric, poorly marginated, multiple infiltrated nodules & plaques or diffuse infiltration; xanthoma-like or dermatofibroma papules; leonin facies and eyebrow alopecia, Granzee zone seen
Nerve lesions	Skin lesions anesthetic early; nerve near lesions sometimes enlarged	Skin lesion anesthetic early; nerve trunk palsies asymmetric; nerve abscesses most common in BT	Anesthetic skin lesions; nerve trunk palsies	Skin lesions usually hypoaesthetic, may be anesthetic; nerve trunk palsies common and frequently symmetric	Hypesthesia a late sign; nerve palsies variable; acral, distal, symmetric anesthesia common
Acid fast bacilli (BI)	3	0-1+	3-4+	4-5+	4-6+
Lymphocytes	3+	2+	1+	1+	0-1+
Macrophage differentiation	Epithelioid	Epithelioid	Epithelioid	Usually undifferentiated; epithelioid foci sometimes present; may show foamy change	Foamy change is the rule, may be undifferentiated in early lesions

Contd...

Contd...

Clinical & Histologic features	Tuberculoid (TT) Borderline	Borderline Tuberculoid (BT) Leprosy	Mild-Borderline (BB) Leprosy	Borderline Lepromatous (BL)	Lepromatous Leprosy (LL)
Langhan's giant Cells	1-3+	2+	-	-	-
Lepromin test	+++	+++	-	-	-
Lymphocyte transformation test	95%	40%	10%	1-2%	1-2%
CD4+/CD8+-T cell ratio in lesions	1.35	1.11	NT	0.48	0.50
M. leprae PGI-1 antibodies	1 + (60%)	2+	2+	3+	3+ (95%)

Reactional States

I. **Type I Lepra reaction/Jopling Type I:**
- *Type IV hypersensitivity* seen in *Borderline* leprosy not in polar form.
- *If precede* therapy then termed *down grading* reaction i.e. towards LL.
- If *after* therapy then termed *Reversal reaction* i.e. towards more tuberculoid.
- Manifestations include classic signs of inflammation within previously involved macules, papules and plaques. Ulnar nerve trunk is the most commonly involved.
- Most characteristic microscopic feature of type I reaction is *Edema.*
- It is associated with increased T cells bearing γ/δ receptors, a unique feature of leprosy.
- MC nerve trunk involved - *Ulnar at elbow*
- **Treatment:**
  - DOC glucocorticoids
  - Clofazimine also given
  - Thalidomide - ineffective.
- If patients are not treated promptly with steroids, irreversible nerve damage may result in as little as 24 h. The most dramatic manifestation is foot drop d/t involvement of common peroneal nerve.

I
- Type I lepra-reaction: Type IV hypersensitivity seen in borderline leprosy
- Type II lepra reaction: Type III hypersensitivity seen in multibacillary (BL and LL) leprosy.

II. **Type II lepra reaction – Erythema Nodosum Leproticum/Jopling Type II:**
- *Type III hypersensitivity* occurs exclusively in BL, LL
- Usually follows therapy (sulfone syndrome) but may precede therapy.
  MC feature - crops of painful erythematous papules that resolve spontaneously in a few days to weeks but may recur.
- Central role in pathobiology: TNF
- **Treatment:**
  - Mild - antipyretics alone
  - Moderate to severe - Ist drug to be used glucocorticoids
    DOC thalidomide
    Clofazimine - More active than in Type I.

III. **Lucio's Phenomenon:**
- **Type III Hypersensitivity** exclusively in diffuse lepromatosis form of LL, usually in untreated patient.
- **Treatment:**
  - Neither glucocorticoid nor thalidomide is effective.
  - Wound care and therapy for bacteremia.

I
Lucio's Phenomenon: Type III *hypersensitivity* – No effective therapy

Complications

- MC complication of leprous neuropathy is *plantar ulceration* particularly at metatarsal heads.
- *Nerve abscess:*
  - MC site is ulnar nerve.
  - Treatment is rapid surgical decompression.

Diagnosis

- Biopsy of advancing edge of lesion in TT but in LL, biopsy of normal skin is also taken.
- In lepromatous leprosy, nodules plaques and indurated areas are optimal biopsy site.
- In tuberculoid leprosy, lesional areas preferably the advancing edge must be biopsied.
- If M antibodies to PGL-1 are found in 95% of patients with untreated lepromatous leprosy and <65% of tuberculoid leprosy patients.
- Hyperglobulinemia in LL.
- *Lepromin test* – Type IV delayed hypersensitivity which is biphasic.
- *Early reaction of Fernandez* – read in **24 - 48 hours (analogous to tuberculin reaction).**
- *Late reaction of Mitsuda* – peak in **4 weeks.** More meaningful.
- It is of little diagnostic value but has more prognostic importance.

I

Lepromin test: Type IV hypersenstivity

Treatment

Form of leprosy	More intensive regimen	WHO recommended regime
i. Tuberculoid (paucibacillary)	Dapsone 100 mg/d × 5 years	Dapsone 100 mg/d + Rifampin 600 mg/month for 6 months
ii. Lepromatous (Multibacillary) or > 6 skin lesions	Rifampin 600 mg/d for 3 years + dapsone 100 mg/d indefinitely	Dapsone 100 mg/d + Clofazimine 50 mg/d and Rifampin 600 mg + clofazimine 300 mg monthly for 1year

- Single lesion paucibacillary leprosy - Single dose of ROM - rifampin, ofloxacin, minocycline.

Remember: Mycobacterium lepraemurium is a causative agent of rat leprosy.

Mycobacteria Tuberculosis

I

Mycobacterium tuberculosis complex:
Group of closely related mycobacteria that can cause tuberculosis, includes:
- M. tuberculosis
- M. bovis
- M. africanium
- M. caprae
- M. pinnipedi
- M. microti
- M. carnetti

Morphology

- Mammalian tubercle, isolated by Koch is stained by Ziehl - Neelsen method or by **fluorescent dyes (auramine O, rhodamine).** They cannot be classified as Gram positive or negative.
- When strained with carbol fuschin they resist decolorization by 20% H_2SO_4 and absolute alcohol for 10 minutes. Hence acid and alcohol fast.
- Acid fastness is due to unsaponifiable wax (mycolic acid) or to a semipermeable membrane.
- It has thick cell wall; shows spheroplast and L forms.

Constituents of Tubercle Bacilli

a. **Lipids:** Mycobacteria are rich in lipids, which include mycolic acid, waxes and phosphatides. Muramyl dipeptide complexed with mycolic acid is involved in granuloma formation, while phospholipids induce caseous necrosis.

b. **Proteins:** Mycobacterium contains several proteins that elicit the tuberculin reaction.

c. **Polysaccharides:** Mycobacterium contains variety of polysaccharides. These polysaccharides can induce immediate hypersensitivity.

I

Stains for Mycobacterium tuberculosis:
- Ziehl-Neelsen stain
- Auramine O rhodamine

M. bovis stains more uniformly

Culture

- Generation time 14-15 hours.
- Colonies appear in about **2 weeks (may take up to 8 weeks).**
- Grows **luxuriantly in culture (Eugonic)** and addition of 0.5% glycerol and CO_2 improves its growth but has no effect on **M. bovis** (causative agent of bovine tuberculosis) **which is dysgonic (grows sparsely).**
- Solid medium most widely employed for routine culture is Lowenstein - Jensen (LJ) medium without starch.
- Liquid media are not generally used routinely, but used for sensitivity testing, chemical analyses and preparation of antigens and vaccines. e.g. Middle brook, MGIT system.
- In liquid culture grow as twisted rope like colonies *(mimicing serpentine cords).*
- *M. tuberculosis* is an obligate aerobe, while *M. bovis* is microaerophillic in primary isolation.

- When incorporated in soft agar media M. tuberculosis grows on the surface whereas M. bovis grows as a band a few mm below the surface.
- *Virulent strain form long serpentine rods in liquid media while avirulent strain grow in dispersed manner. Cord formation is correlated with virulence.* ... *Jawetz 27/e, p 312*
- A cord factor (trehalose 6.6′ dimycolate) has been extracted from virulent bacilli. It inhibits migration of leukocytes causes chronic granuloma and can serve as a immunologic adjuvant.

Biochemical reaction	Positive in	Negative in
Niacin test : **N**	Human tubercle	Bovine tubercle
Aryl sulphatase : **A**	Only with atypical mycobacteria	
Neutral red test : **N**	Virulent strain of tubercle	Avirulent strain
Peroxidase test : **P**	Tubercle bacilli	Atypical mycobacteria
Catalase test : **C**	Most atypical mycobacteria	Weakly positive in tubercle
Nitrate reduction test : **N**	*M. tuberculosis*	*M. bovis*

I
- Tubercle bacilli are non-motile, non-sporing, non-capsulate, straight or slightly curved
- L J medium: Egg glycerol based medium to which malachite green dye is added, so as to inhibit the growth of other contaminating bacteria and to provide contrasting colour.

- *Catalase and peroxidase activities are lost when tubercle bacilli become INH resistant.*
- Urea test is positive in *M. tuberculosis, M. bovis* and most of the atypical mycobacteria except MAIC complex.
- Mycobacteria are susceptible to alcohol, formaldehyde, glutaraldehyde and, to a lesser extent, hypochlorites and phenolic disinfectants.

Virulence factors:

- **Kat-G gene:** encodes for oxidase, catalase enzyme.
- **rpoV:** main sigma factor initiating transcription of several genes.
- **Erp gene:** encodes for protein required for multiplication.
- **Strains of Beijing/w genotype family.**

Antigenic Property

- Group specificity is due to polysaccharide while type specificity is due to protein antigen.
- Antibodies are not useful for diagnosis and immunity.

Pathogenicity

- Majority of inhaled bacilli are trapped in upper airways, 10% reach the alveoli. There antigens of mycobacteria processed through APC activates T-cell. Activated T-cells secrete IFN γ which together with calcitriol, activate macrophages. Macrophage engulf bacteria but does not kill them. ...Greenwood 18/e, p214
- It is due to escape killing by macrophages and induction of type IV hypersensitivity.
- *Following factors contribute in pathogenesis:*
  - Cord factor
  - Lipoarabinomannan
  - Complement system
  - *M. TB* heat shock protein.
- Risk of ***acquiring*** infection is determined mainly by *exogenous* factors while risk of developing ***disease*** depends largely on *endogenous* factors.
- Most potent risk factors - HIV coinfection.

I
Group specificity is due to polysaccharide while type specificity is due to protein antigen.

Clinical Features

- Divided into two categories: Pulmonary and extrapulmonary TB.
  1. **Pulmonary TB:** Divided into two:
     a. ***Primary Disease:*** Usually localized in middle and lower zones.

     Primary focus is usually peripheral in subpleural region and is accompanied by draining lymphatics, inflamed regional lymph nodes which are collectively called

Note: When the bacilli enters through mouth as in case of M. bovis primary complex involve the tonsil and cervical nodes or the intestine often the ileocaecal region and the mesenteric nodes. In occupational exposure (pathologist) primary focus may be in skin called as prosector's wart.

Depending on the host immune response development of complex can follow healing by fibrosis/calcification; cavitation or progressive primary TB in form of consolidation; obstructive emphysema or atelactasis; TB bronchitis; miliary TB; occult hematogenous dissemination to apex of lung **(Simons Focus)**.

b. ***Post primary disease*** (adult type or reactivation or secondary tuberculosis or chronic pulmonary TB).
   - Usually localized to apical and posterior segments of upper lobe due to high O_2 concentration **(Puhl's Lesion)**.
   - **MC hematologic** finding - mild anemia and leucocytosis
   - Infraclavicular lesion is called *Assman's Focus*.

Extrapulmonary TB

- **MC site is lymph node** (**MC** *cervical and supraclavicular*).
- **Other Sites include:**
  - Pleura in the form of pleural effusion and empyema.
  - Genitourinary tract (culture negative pyuria in acidic urine).
  - Skeletal TB (**MC** site spine, hip, knee).
  - TB meningitis (paresis of cranial nerves especially *ocular*, is frequent finding).
  - **GI TB (MC** site terminal ileum and caecum).
  - Tuberculous pericarditis (**MC** cause of chronic constrictive pericarditis).

I

Most common site of TB: Lung
Most common site of extrapulmonary TB: Lymph node

Diagnosis

Specimen – sputum is best collected in the ***morning*** before any meal *(3 sample)*.

i. **AFB microscopy:** smear should be prepared from thick purulent part of sputum.
   - At least **10000 AFB** should be present per ml of sputum for demonstrating in direct smears. Positive report can be given only if >2 typical bacilli have seen.
   - **Fluorescent microscopy** (stained with auramine phenol or auramine rhodamine fluorescent dye and examined under UV illumination) screen smear *rapidly* in comparison of Ziehl–Neelsen method.
   - Concentration method for microscopy can also used, e.g. Petroff's method using NaOH solution is widely used.

I

- Petroff Method: Most widely used method for sputum culture. Here sputum is mixed with 4% NaOH, neutralized with potassium dihydrogen orthophosphate and centrifuged. The deposit is then inoculated on LJ medium.

Slide reporting

The number of bacilli seen in a smear reflects disease severity and patient infectivity. Therefore, it is important to record the number of bacilli seen on each smear. The standard method of reporting using 1000 × magnification is as:

Number of bacilli	Rresult reported
No AFB per 100 oil immersion firlds	0
1–9 AFB per 100 oil immersion fields	scanty (or number AFB seen)
10–99 AFB per 100 oil immersion fields	+ (1 +)
1–10 AFB per oil immersion field	++ (2 +)
> 10 AFB per oil immersion field	+++ (3 +)

Laboratory technicians should examine both the sputum samples from each TB suspect. It is advised that the smear examined by one microscopist should not exceed 20 per day as visual fatigue leads to a deterioration of reading quality.

ii. **Culture:**
   - Very ***sensitive diagnostic*** technique can detect even 10 to 100 bacilli per ml.
   - LJ and Middle brook 7H10/7H11 are selective media.
   - Negative report is given, if no growth occur after 8-12 weeks.
   - Slow growing, nonpigmented niacin positive AFB is taken as *M. tuberculosis*.
   - Liquid media with radiometric growth detection (e.g. BACTEC 460) and nucleic acid probes, enables results to be given in 2-3 weeks.

iii. Nucleic acid technology:

- PCR and Ligase chain reaction are used as diagnostic technique.
- RFLP and 15 fingerprinting used for epidemiological typing of strain.
- Xpert MTB/RIF assay: Simultaneously detect TB and rifampicin resistance in <2h and has minimal biosafety and training requirements. Who recommends its worldwide use as initial diagnostic test in adults and children.

iv. Immunodiagnosis:

a. Tuberculin skin test

- Demonstration of hypersensitivity to tuberculin protein (tuberculin test/Mantoux intradermal test) is a standard procedure.
- 1 purified protein derivative (PPD) = 50000 tuberculin units per milligram.
- WHO advocates PPD tuberculin known as - RT 23 with Tween 80.
- ***Routinely*** 1 TU used.
- ***Clinically*** 5 TU used.
- Read after 72 hours in which induration is measured in horizontal transverse diameter.
  - > 10 mm positive, < 5 mm negative.
  - < 6 and > 15 mm have more risk of developing TB.
- Positive tuberculin test indicates exposure to bacilli *(infection, immunization)* with or without clinical disease. So persons who have never had contact with bacilli are tuberculin negative.
- Used as aid in diagnosing active infection in infants and young children; measure prevalence of infection; to select susceptibles; as an indicator of successful vaccination.
- Tine Multiple puncture test and heaf test is used for screening and surveys.

b. IFN γ Assay:

- Two in vitro assays that measure T cell release of IFN γ in response to stimulation with highly TB specific (IGRA) antigens ESAT-6 and CFP 10, are available by the name of **T-SPOT TB** (an enzyme linked immunospot assay) and **Quanti FERON-TB Gold** (whole blood ELISA).
- IGRA are preferred over tuberculin test (TST) for persons above 5 years of age, in children under 5 years TST is preferred.

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Poncet disease:
Reactive arthritis in tuberculosis.
Spina ventosa: TB of phalanges
Caries sicca: TB of shoulder

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- Mantoux test: < 6 mm and > 15 mm have more risk of developing TB.
- WHO has banned use of tubercular IgM ELISA due to very high false positive rate, thus over prescription of anti-tubercular therapy.

Treatment

ATT is given: First line drugs are:

Drugs	Daily dose	Dose in DOTS	Thrice weekly dose
H. Isoniazid	5 mg/kg	600 mg	10 mg/kg
R. Rifampein	10 mg/kg	450 mg	10 mg/kg
Z. Pyrazinamide	25 mg/kg	1500 mg	35 mg/kg
E. Ethambutol	15 mg/kg	1200 mg	30 mg/kg
S. Streptomycin	15 mg/kg	750 mg	15 mg/kg

- **MDR Strains:** Strains resistant to isoniazid and rifampicin with or without resistance to additional drugs
- **XDR Strains:** Strains resistance to at least isoniazid, rifampicin, any tluoroquinolones and any injectable agent.

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- Xpert MTB/RIF based on amplification of mycobacterial nuclei acid has emerged as initial diagnostic test.
- This test has a sensitivity of 98% among AFB positive cases and 70% among AFB negative specimens.

Prevention

- *BCG vaccine:* Live attenuated vaccine derived from attenuated bovine strain of *tubercle bacilli.*
  - Normal saline is diluent
  - Dose of 0.05 ml for age < 4 week and 0.1 ml for > 4 weeks should be given intradermal (subcutaneous administration may lead to abscess).

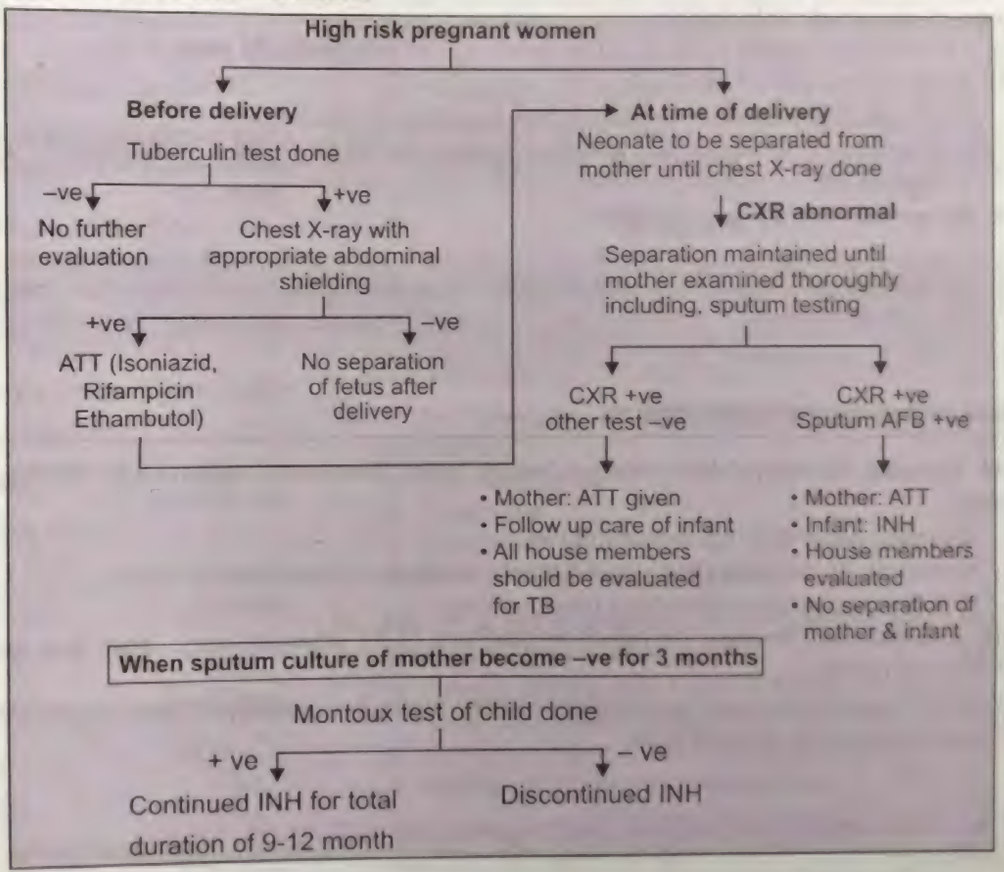
- Neonate of infected mother: Give INH resistant BCG + INH prophylaxis for 6 weeks.
- *Chemoprophylaxis (preventive treatment):* INH for 1 year or INH plus ethambutol for 9 months.

Perinatal TB

..... Nelson 17/e, 967, 971

- MC sign and symptoms of congenital TB are respiratory distress, fever, hepatic or splenic enlargement, poor feeding, lethargy, irritability, lymphadenopathy, abdominal distension, failure to thrive, ear drainage and skin lesions.
- Symptoms most commonly begin by 2nd or 3rd week of life.
- A positive acid fast stain of an early morning gastric aspirate from newborn usually indicate TB.
- Most important clue for rapid diagnosis is maternal or family history of TB.
- Most effective way of preventing congenital TB is appropriate testing and treatment of mother and other family members.

Approach to Peri-natal Tuberculosis



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- MDR strain (Multidrug resistant strain): Strain resistant to isoniazid and rifampicin with or without resistance to additional drugs.
- XDR-TB (Extensively drug resistant TB): MDR Strain that is resistant to, fluoro-quinolone and one of the second line injectables.
- Mutation to drug reistance occurs at a rate of about one mutation every 10^6 cell division.

ATYPICAL MYCOBACTERIA

- Also known as Unclassified or Environmental or Opportunistic mycobacteria.
- It includes mycobacterial species other than *Mycobacterium tuberculosis* complex and *M. leprae*, hence, called as Paratubercle or tuberculoid or MOTT (mycobacteria other than tubercle) bacilli.
- It is divided into 4 groups based on colony pigmentation (Runyon's classification).

Group I – Photochromogens - Produce pigment only in light.

- Slow growing though growth is faster than that of tubercle bacilli. It includes:
  a. **M. kansasii** – cause chronic pulmonary disease in old persons with pre-existing lung disease.
  b. **M. marinum** – cause warty skin lesion *(swimming pool granuloma).*
  c. M. simiae

Group II – Scotochromogens - Form pigment even in dark.

a. **M. scrofulaceum** – cause scrofula (cervical adenitis) in children.
b. **M. gordonae** – called as *Tap water scotochromogen.*
c. **M. szulgai** – scotochromogen and photochromogen.

Group III – non-photochromogens - Not form pigment even in light.

- Colonies may resemble those of tubercle bacilli.
  a. **M. intracellulare** – also known as *Battey bacillus.*
  b. **M. avium** – MAIS complex (i.e. avium, intracellulare, scrofulaceum) cause lymphadenopathy, pulmonary lesion and disseminated disease particularly in **AIDS** patient.
- **Other non-chromogens are:**
  - *M. ulcerans, M. shinshvense, M. paratuberculosis, M. xenopi, M. malmoense, M. sylvaticum, M. lapraemurium, M. terrae, M. hemophilum, M. genevense*

Group IV – Rapid growers

- Colonies appear within seven days. They can be photochromogenic, scotochromogenic, non-chromogenic. It includes:
  a. Chromogenic saprophytic rapid growers, e.g. *M. smegmatis, M. phlei*
  b. ***M. fortuitum and M. chelonae*** – Not form pigment.
     - Cause chronic abscesses (may follow injection of vaccines).
     - Pulmonary lesion of *M. fortuitum* cannot be distinguished radiologically from typical TB.
  c. ***M. vaccae*** – Immunomodulator.

> **Remember:** M. chelonei also called as turtle tubercle bacillus while M. fortuitum also called do frog tubercle bacillus.

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Common site of infection
- M. kansasii: Lung
- M. marinum: Skin, swimming pool granuloma
- M. avium: AIDS patient
- M. scrofulaceum: Scrofula

SKIN PATHOGEN MYCOBACTERIAS

- ***M. ulcerans:*** Exclusive skin pathogen which cause *Buruli ulcer* usually seen on legs or arms.
  - Infection occur through minor injuries.
  - Grows on LJ medium slowly in 4-8 weeks at critical temperature (30-33°C).
  - It is the *only mycobacteria* which produce *toxin.*
- ***M. marinum (M. balnei):*** Its infection (but not of *M. ulcerans*) may cause low grade *tuberculin reaction.*
- Regional lymph nodes are not involved as they multiply optimally at skin temperature.
- Drug resistance in mycobacteria.

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Buruli ulcer: Caused by M. ulcerans

Multiple Choice Questions

M. TUBERCULI

1. **Basanti, 29 year aged female from Bihar present with active TB. She delivers baby. All of the following are indicated except:** [AI 01]
   a. Administer INH to the baby
   b. Withhold breastfeeding
   c. Give ATT to mother for 2 years
   d. Ask mother to ensure proper disposal of sputum

2. **Tuberculin test denotes:** [AI 00]
   a. Previous or present sensitivity to tubercle proteins
   b. Patient is resistant to TB
   c. Person is susceptible to TB
   d. Protective immune status of individual against TB

3. **Which of the following regarding the Interferon-gamma release assays used for the diagnosis of tuberculosis is correct?:** [AI 2012]
   a. 1st generation Quantiferon-TB used ESAT-6
   b. 2nd generation Quantiferon-TB (gold) used ESAT-6 and CFp-10
   c. These tests can distinguish between *M. tuberculosis* and *M. bovis*
   d. None of the non-tubercular mycobacteria give a positive reaction with this test

4. **Collection of urine sample of a patient of TB kidney is done:** [AIIMS 00]
   a. 24 hrs urine
   b. 12 hrs urine
   c. In early morning
   d. Any time

5. **True about Mantoux test:** [PGI 03]
   a. < 5 mm always +ve
   b. Usually -ve after treatment
   c. Positive reaction in children < 2 is not improtant like in adult
   d. Usually red after 48-72 hours
   e. False +ve in post measles state

6. **True regarding *Mycobacterium tuberculosis* is:**
   a. Produces visible colonies in 1 week time on Lowenstein Jenson media
   b. Decolorised by 20% sulphuric acid
   c. Faculatative aerobe
   d. Niacin positive [PGI 02]

7. **Which of the following are acid fast positive with 20% sulphuric acid:** [PGI 02]
   a. *M. avium*
   b. *M. leprae*
   c. *M. tuberculosis*
   d. *Nocardia*
   e. Rhizopus

8. **Selective media for TB bacilli is:** [PGI 01]
   a. NNN media
   b. Dorset media
   c. LJ media
   d. Nutrient agar
   e. Mac'Conkey media

9. **Not easily culturable but well viable and used in epidemiology are:** [PGI 00]
   a. *Staph.*
   b. *Mycobacterium TB*
   c. *E. coli*
   d. *Salmonella*

10. **True about tuberculin test are all except:** [PGI 98, 01]
    a. Recent conversion in adult is an indication for ATT
    b. INH prophylaxis is started if the test is positive
    c. No risk in negative
    d. May be false negative in immunocompromised patients

11. **Cavitation is most often seen in:** [AI 2011]
    a. Mycoplasma pneumonia
    b. Tuberculous pneumonia
    c. Streptococcal pneumonia
    d. Staphylococcal pneumonia

M. LEPRAE

12. **Leprosy affects all the following except:** [AI 07]
    a. Testes
    b. Ovaries
    c. Eyes
    d. Nerves

13. **In the management of leprosy, Lepromin test is most useful for:** [AI 03]
    a. Herd immunity
    b. Prognosis
    c. Treatment
    d. Epidemiological investigations

14. **Which of the following is true regarding globi in a patient with lepromatous leprosy:** [AI 02]
    a. Consist of lipid laden macrophages
    b. Consist of macrophages filled with AFB
    c. Consist of neutrophils filled with bacteria
    d. Consist of activated lymphocytes

15. **The main cytokine, involved in erythema nodosum leprosum (ENL) reaction, is:** [AIIMS 06]
    a. Interleukin-2
    b. Interferon - gamma
    c. Tumor necrosis factor - alpha
    d. Macrophage colony stimulating factor

16. **The following drug is not used for the treatment of type II lepra reaction:** [AIIMS 06]
    a. Chloroquine
    b. Thalidomide
    c. Cyclosporine
    d. Corticosteroids

17. **The following test is not used for diagnosis of leprosy:** [AIIMS 06]
    a. Lepromin test
    b. Slit skin smear
    c. Fine needle aspiration cytology
    d. Skin biopsy

18. **Which of the following statement about lepromin test is not true:** [AIIMS 07, 06]
    a. It is negative in most children in first 6 months of life
    b. It is a diagnostic test
    c. It is an important aid to classify type of leprosy disease
    d. BCG vaccination may convert lepra reaction from negative to positive

19. **Under leprosy eradication program the management of single lesion is:** [AIIMS 02]
    a. Single dose of Rifampicin and Dapsone
    b. Rifampicin and Dapsone for 6 months
    c. Rifampicin, Ofloxacin and Minocycline single dose
    d. Rifampicin and Monocycline for 6 months

20. **The characteristic finding in a case of leprosy is:**
    a. Culture test is positive in 2-3 months in LJ media
    b. Long contact with tuberculoid leprosy can transmit the disease [AIIMS 98, 2000]
    c. CMI is seen in Lepromatous leprosy
    d. Macule lesion heals spontaneously

21. **Exacerbation of lesions in patients of borderline leprosy is seen in:** [PGI 01]
    a. ENL (erythema nodosum leprosum)
    b. Lepra reaction type I
    c. Jarisch-Herxheimer reaction
    d. Resolving leprosy

ATYPICAL MYCOBACTERIA

22. **Antibodies against PGL - 1 are seen in:**
    a. M. leprae [AIIMS 2013, AI 2011, DNB 2012]
    b. M tuberculosis
    c. Borrelia
    d. Brucella

23. **Which one of the following statement is true regarding pathogenicity of Mycobacteria species:** [AI 06]
    a. *M. tuberculosis* is more pathogenic than *M. bovis* to humans
    b. *M. kansasii* can cause disease indistinguishable from tuberculosis
    c. *M. africanum* infection is acquired from environmental source
    d. *M. marinum* is responsible for tubercular lymphadenopathy

24. **The most common focus of Scrofuloderma is:**
    a. Lung b. Lymph node [AI 96, 04]
    c. Larynx d. Skin

25. **True about mycobacterium other than tuberculosis:**
    a. Causes disseminated infection [AIIMS 08]
    b. Occurs in persons with normal immunity
    c. Causes decreased efficacy of BCG due to cross immunity
    d. Person to person transmission is seen

26. **Scotochromogens are:** [PGI Dec 2008]
    a. *Mycobacterium gordonae*
    b. *Mycobacterium marinum*
    c. *Mycobacterium Intracellulare*
    d. *Mycobacterium avium*
    e. *Mycobacterium kansasii*

27. **Which of the following are photo chromogens?**
    a. *M. kansasii* b. *M. scrofulosorum*
    c. *M. marinum* d. *M. TB*
    e. *M. leprae* [PGI Dec 2008]

28. **Which of the following is a slow grower:** [AIIMS May 2013]
    a. *M. kansasii* b. *M. chenolae*
    c. *M. fortuitum* d. *M. abscessus*

29. **Rapid growing non-tuberculous mycobacteria causing lung infection are all except:** [AIIMS Nov 2013]
    a. *M. kansasii*
    b. *M. chelonae*
    c. *M. fortuitum*
    d. *M. abscessus*

Explanations and References with Illustrative Answers

1. **Ans. (b) Withhold breastfeeding** *Ref. Nelson 17/e, p 971*

If the mother is suspected of having active disease or detection of an acid fast bacilli in sputum shows evidence of current tuberculosis disease, besides giving ATT to mother certain additional steps are necessary to protect the infant:

a. **INH therapy**

"INH therapy for newborns is so effective that separation of mother and infant is no longer considered mandatory."

- Separation should done (until mother become non-infectious) only if:
  - Mother is so ill so as to require hospitalization.
  - She is expected to become non-adherent with her treatment.
  - There is strong suspicion that she has drug resistant tuberculosis.
- INH treatment of infant should be continued until the mother has been shown to be sputum culture negative for at least 3 months.

b. **Appropriate treatment of mother and other family members.**

- Though there is controversy in the question as according to 'Some books' Breastfeeding is contraindicated and isolation of infant from the mother having active TB should done.
- But as all other three options are totally correct and as Indian child must have breastfeeding, I have to go with Nelson only.

2. **Ans. (a) Previous or present sensitivity to tubercle proteins**

Ref. Park 21/e, p 168, 22/e p 172; Ananthanarayan 8/e, p 356, 9/e, p 354

- Tuberculin test denotes Type IV hypersensitivity to tuberculoprotein but not cellular immunity.
- Positive tuberculin test indicates exposure to bacilli either in the form of infection or immunization with or without clinical disease.
- It does not indicate that active infection is present (except in infants and young children).
- It does not indicate whether person is able to mount immune response against bacilli or not **(as in lepromin test).**
- It does not indicate resistance or susceptibility to TB **(as in Schik's test).**

3. **Ans (b) 2nd generation Quantiferon-TB (gold) used ESAT-6 and CFp-10** *Ref. Harrison 18/e, p 1352; 1370; 19/e, p 1113*

IFN γ release Assays (IGRA)

- In vitro assays that measure T-cell release of IFN γ in response to stimulation with the highly TB specific antigen ESAT-6 and CFP-10. Presently two such assays are available:
  (a) **T. SPOT TB:** An enzyme linked immunosorbent assay.
  (b) **Quantiferon-TB Gold:** Whole blood enzyme linked immunosorbent assay.
- IGRA are more specific than the TST (*Mantoux test*) as they are negligibly effected by previous BCG vaccination and sensitization by non-tubercular mycobacteria.
- IGRA require blood to be drawn from patient and delivered to lab where test is done, in contrary to tuberculin test in which PPD is injected. So, these test can be repeated without boosting response.
- In near future IGRA are supposed to replace tuberculin skin test as preferred skin test for detection of latent tuberculin infection.
- M. marinum, M. kansasii, M. szulgai also have ESAT 6 and CFP 10 and may cause false positive reactions.
- Currently IGRA is preferred over TST in population above 5 years of age. In children under 5 years TST is preferred and in individuals who are at high risk of progression to active TB either test can be used.

Note: In 2013 WHO has banned IgM ELISA for tuberculosis, and described that test as one of the most misused test.

- Though in previous reports. IGRA's found to be more specific than TST, however recent studies suggest that IGRA may not perform well in serial testing. So WHO does not recomend the replacement of TST by IGRA in low income group countries like India. *Ref. Harrison 19/e, p 1114*

4. **Ans. (c) In early morning** *Ref. Harrison 19/e, p 1011, 18/e, p 1347*

Genitourinary TB

- Accounts for 15% of all extrapulmonary cases.
- Urinary frequency **(MC)**, dysuria, hematuria and flank pain are common presentation.

Diagnosis: i. Urinalysis gives abnormal result in 90% of cases.
ii. Culture of three morning urine specimens yields a definitve diagnosis in nearly 90% cases. Culture negative pyuria in acidic urine raises the suspicion of TB.
iii. IVP - Calcification, ureteral stricture and Hydronephrosis may seen.

Genital TB:

Female > male
MC site in **female** – Fallopian tube
MC site in **male** – Epididymis
Genitourinary TB respond well to chemotherapy.

5. **Ans. (d) Usually red after 48-72 hours** *Ref. Ananthanarayan 8/e, p 356, 9/e, p 354-355; Park 21/e, p 168; 22/e p 172*

- Tuberculin test is the only means of estimating prevalence of infection in a population.
- **It is of three types:**
  - Mantoux intradermal test
  - Heaf test - for testing large groups
  - Tine multiple puncture test - not recommended.
- Standard PPD (purified protein derivative) contains 50,000 tuberculin units per milligram.
- WHO advocates PPD tuberculin known as PPD RT-23 with Tween 80.
- For routine testing 1 TU used, while clinically 5 TU used.

Mantoux Test

- 0.1 ml of 1 TU injected intradermally
- Examined after 72 hours and induration is measured horizontally in mm and is interpreted as:

> 10 mm	:	Positive
< 6 mm	:	Negative
6-9 mm	:	Doubtful that is the reaction may be due to M. tuberculosis or atypical mycobacteria
> 20 mm	:	Strong reactors – Greater chance of developing TB
< 5 mm	:	More risk of developing TB than those with 6-9 mm induration

- Positive reaction indicates that person has got previous exposure to M. tuberculosis but it does not prove that person is suffering from disease.
- Positive reaction is significant in younger age groups (< 2 year).
- ***Negative test cannot taken as exclusion of TB*** since dermal hypersensitivity to tuberculin can also be lost in immunosuppressive states which gives ***false -ve result*** e.g. Malignancy, Hodgkin's disease, post measles state, corticosteroid therapy.
- Repeat test may appear to be negative or exerts a booster effect.
- Positive tubercullin test may occasionally revert to negative upon INH treatment.
- ***After infection patient becomes tuberculin positve in 3-6 weeks (= I.P of TB).***
- ***After 8 weeks of BCG vaccination it becomes positive.***

6. **Ans. (d) Niacin positive** *Ref. Ananthanarayan 8/e, p 348 - 349, 9/e, p 347*

Mycobacteria tuberculosis is:

- Gram positive, non-motile, noncapsulated, non sporing.
- Obligate aerobic.
- Acid fast (resist decolorization by 20% H_2SO_4) and alcohol fast.
- Generation time 14-15 hours.
- Colonies appear slowly in about 2 weeks and may sometimes take up to 8 weeks.
- Niacin, Neutral red, Nitrate reduction test positive (3N is positive).
- Peroxidase and Urease test is also positive.

7. **Ans. (c) M. tuberculosis** *See below*

Features	M. tuberculosis	M. leprae
Acid fastness	Resist decolorization by 20% H_2SO_4	Resist decolorization by 5% H_2SO_4
Alcohol fastness	Present	Absent
Culture	Possible	Not possible
Niacin	Positive (also some strain of *M. microti*)	Negative
Generation time	14-15 hours	12-13 days

Remember: Nocardia resist 1-4% of sulphuric acid (weakly acid fast).

8. **Ans. (b) and (c) Dorset media and LJ media** *Ref. Ananthanarayan 8/e, p 348, 9/e, p 346-347*

Media for M. Tuberculosis

Solid	Liquid
• Lowenstein Jensen media *(most widely used)*	• Dubos contain Tween 80
• Dorset egg media	• Middle Brook's
• Loeffler's media	• Proskauer and Beck's
• Pawlowsky media	• Sula's and Sautan

- Selective agent inhibiting other bacteria in LJ media is Malachite green.
- Human tubercle bacilli do not grow in presence of para-nitrobenzoic acid.
- Traces of fatty acid is toxic for tubercle bacilli in culture media.
- Optimum pH for M. tuberculosis: 6.4-7.0.

Other Options:

NNN media – For Leishmania donovani
Nutrient agar – Simple media
MacConkey media – Differential as well as indicator media for lactose and non-lactose fermenters.

9. **Ans. (b) Mycobacterium TB** *Ref. Ananthanarayan 8/e, p 348, 9/e, p 346*

- **S. aureus:** grow rapidly on ordinary media within a temperature range of 10-42°C.
- **E. coli:** good growth occurs on ordinary media.
- **Salmonella:** grow readily on simple media over range pH 6-8 and temperature 15-41°C.
- **M.TB:** grow slowly, colonies appear in 2-8 weeks. Culture remain viable at room temperature for 6-8 months and may be stored up to 2 year at –20°C.

10. **Ans. (c) No risk in negative** *Ref. Park 21/e, p 168, 22/e p 172; KDT 6/e, p 749*

Risk of developing TB is more in patient with Mantoux result < 6 mm i.e. negative.

Indication of Chemoprophylaxis

i. Contacts of open cases who show recent Mantoux conversion.
ii. Children with positive Mantoux and a TB patient in the family.
iii. Neonate of tubercular mother.
iv. Patient of leukemias, diabetes, silicosis or those who are HIV positive but are not anergic or are on corticosteroid therapy who show a positive Mantoux.
v. Patient with old inactive disease who are assessed to have received inadequate therapy.

Now drug used for chemoprophylaxis:

- Combination of Isoniazid (5 mg/kg) and Rifampicin (first line)
- Isoniazid for 12 months (second line)

In a country like India chemoprophylaxis is not recommended because of limited resource and high prevalence.

11. **Ans. (b) Tuberculous pneumonia** *Ref. Harrison 17/e, p 1622*

Pneumonia pattern	Etiologic organism
Lobar distribution	Streptococcus pneumoniae
Bulging fissure	Klebsiella
Cavitation	Tuberculosis
Pneumatocele	Staphylococcus
Alveolar nodules	Varicella, Tuberculosis
Pulmonary edema	Viral/Pneumocystis pneumonia

12. Ans. (b) Ovaries *Ref. Harrison 19/e, p 1122, 18/e, p 1360; International Journal of Leprosy, Vol. 71, No. 2, p 101-105*

Mycobacterium leprae grows best in cooler (the skin, peripheral nerves, anterior chamber of the eye, upper respiratory tract, and testes), sparing warmer areas of the skin (the axilla, groin, scalp, and midline of the back).

Thus, ovary is the answer of exclusion.

Complications of M. leprae

- *Eye:* Uveitis, cataracts, glaucoma, corneal insensitivity and lagophthalmus.
- *Testes:* Orchitis followed by impotence.
- *Nerve abscesses:* Mostly seen in BT form, **ulnar nerve is most frequently involved.**
- *Extremities:* Plantar ulceration (most frequent complication of leprous neuropathy), footdrop, Charcot's joints.
- *Nose:* Epistaxis, saddle-nose deformity or anosmia.

13. Ans. (b) Prognosis *Ref. Ananthanarayan 8/e, p 368, 9/e, p 367*

Lepromin test:

- It is intradermal test which shows delayed type IV hypersensitivity.
- It is biphasic:
  - **a. Early reaction of Fernandez:** Read in 24-48 hours (analogous to tuberculin reaction)
  - **b. Late reaction of Mitsuda:** – Peak in 4 weeks
    – It is more meaningful.
- It distinguishes between persons who can mount CMI against lepra bacillus antigens and those who cannot.
- So, ***finally lepromin test is of little diagnostic value but has more prognostic value.***
- ***It is used to:***
  - Classify the lesions of leprosy
  - To assess prognosis and response to treatment
  - To assess resistance of individual to leprosy
  - To verify the identity of candidate lepra bacilli.
- **Antigen used in lepromin test:**
  - Modern antigens contain 4×10^7 lepra bacilli per ml.
  - Standard lepromins derived from armadillo derived lepra bacilli (lepromin A) replacing human derived human lepromin H.

14. Ans. (b) Consists of macrophages filled with AFB *Ref. Ananthanarayan 8/e, p 364; 9/e, p 364*

- Acid fast lepra bacilli may lie extracellularly or intracellularly in large undifferentiated histiocytes (type of macrophage) called as **Virchows 'Lepra cells' or 'Foamy cells'.**
- Bacilli are bound together by lipid like substance the glia forming masses known as **'Globi'.**

15. Ans. (c) Tumor necrosis factor - alpha *Ref. Harrison 19/e, p 1125, 18/e, p 1363; KDT 6/e, p 756*

TNF plays a central role in pathobiology of ENL.

Type I lepra reaction	Type II Lepra reaction
• Downgrading or reversal reaction	• Erythema nodosum leproticum
• Type IV hypersensitivity	• Type III hypersensitivity
• TNF play a central role	• IFNγ and IL-2 are main cytokines involved
• Edema is characteristic microscopic feature	• Vasculitis and panniculitis are seen
• **Treatment: DOC** – Glucocorticoid Other drugs: – Clofazimine – Chloroquine – Analgesics Thalidomide is Ineffective	• **DOC** – Thalidomide Other – Clofazimine – Chloroquine – Glucocorticoids – NSAIDs & antibiotics

16. Ans. (c) Cyclosporine *Ref. Harrison 19/e, 1125, 18/e, 1366; KDT 6/e, p 756*

Cyclosporine has no role in lepra reaction.

For more details, refer above answer.

17. **Ans. (a) Lepromin test** *Ref. Ananthanarayan 8/e, p 368, 9/e, p 367; Harrison 19/e, p 1126*

Note: PCR of the skin for M. leprae although positive MLL and BL disease negative result in 50% of tuberculoid cases.

Lepromin test is not used for diagnosis.

18. **Ans. (b) It is a diagnostic test** *Ref. Ananthanarayan 8/e, p 368, 9/e, p 367*
Already explained

19. **Ans. (c) Rifampicin, Ofloxacin and Minocycline single dose** *Ref. Harrison 17/e, p 1026, 18/e, p 1365*

Form of Leprosy	WHO recommended regime
i. Tuberculoid (paucibacillary)	Dapsone 100 mg/d unsupervised plus Rifampicin 600 mg/mth supervised for 6 month
ii. Single skin lesion (paucibacillary)	Single dose of **R** 600 mg **R**ifampicin **O** 400 mg **O**floxacin **M** 100 mg **M**inocycline
iii. Lepromatous (multibacillary) >6 skin lesion	Dapsone 100 mg/d plus Clofazimine 50 mg/d unsupervised; and Rifampicin 600 mg plus Clofazimine 300 mg monthly (supervised) for 1 year

20. **Ans. (b) Long contact with tuberculoid leprosy can transmit the disease** *Ref. Harrison 19/e, p 1123, 18/e, p 1360; Dr Arora Textbook of Microbiology 2/e, p 312*

Transmission of Leprosy

- Nasal droplet infection.
- Contact with infected soil and contact with a tuberculoid leprosy case carries a very low risk.
- Insect vectors.
- *Direct dermal inoculation (during tatooing).*
- Household contact with infected lepromatous case.
- Skin to skin contact (though can transmit infection) is ***generally not considered an important route of transmission.***
- Physicians and nurses caring for leprosy patients & the coworkers of these patients are not at risk leprosy.
- According to *Park 18/e, p 254* ***"All patients with active leprosy must be considered infectious".***

Remember:
- Cell mediated immunity (CMI) is present in tuberculoid leprosy and lepromatous leprosy patient do not exhibit CMI to leprosy bacteria Lepra bacilli cannot grow in artificial culture media.
- Park, Ananthanarayan, Harrison do not mention that macules of tuberculoid leprosy heals spontaneously.
- In India maximum leprosy patient are in Bihar > UP.

21. **Ans. (a) and (b) ENL and Lepra reaction I** *Ref. Harrison 19/e, p 1125, 18/e, p 1363*

Reactional state	Type of leprosy
i. Type I lepra reaction	Borderline leprosy
ii. Type II lepra reaction (ENL)	Exclusively in Borderline Lepromatous (BL) and lepromatous leprosy
iii. Lucio's phenomenon	Diffuse lepromatous form of LL
• Jarisch Hexhimer reaction is seen in syphilis patient taking penicillin.	

22. **Ans. (a) M. leprae** *Ref. Harrison 18/e, 1364*

IgM antibodies to PGL-1 are found in 95% of patients with untreated lepromatous leprosy, and 60% of patients with tuberculoid lepresy. Antibodies to PGL-1 may also be seen in exposed individual without clinical leprosy.
Thus PGL-1 serology is of little diagnostic utility in tuberculoid leprosy.

Remember: PGL-1 stands for M. leprae specific phenolic glycolipid found in the cell wall of leprosy bacilli.

23. **Ans. (b) *M. kansasii* can cause disease indistinguishable from tuberculosis** *Ref. Jawetz 27/e, p 318*

"Some atypical bacteria (e.g. Mycobacterium kansasii) produce human disease indistinguishable from tuberculosis."
..... Jawetz, 27/e, 318

Other options:

Option 'a': *M. tuberculosis* and *M. bovis* are equally pathogenic for humans. *.....Jawetz*

Features	M. tuberculosis	M. bovis
Shape	Curved long rod	Straighter, shorter, stouter
Staining	Less uniform	More uniform
O_2 requirement	Obligate aerobe	Microaerophilic
Colonies	Dry, rough, raised, irregular	Flat, smooth, moist, break up easily
Growth	Eugonic	Dysgonic

Options 'c': *M. africanum* is acquired from human and cattles.

Remember:
- M. tuberculosis, M. bovis; M. africanum belongs to TB complex group of Runyons classification.
- Mycobacterium acquired from environmental source:
  - M. **a**vium complex
  - M. **m**almoense (**Mneumonic: AMU** – **A**ligarh **M**uslim **U**niversity)
  - M. **u**lcerans

Option 'd': *M. marinum* causes chronic cutaneous infection when open cutaneous lesion is exposed to colonized water source.

Remember: Atypical bacteria causes lymphadenitis are: M. avium, M. intracellulare, M. malmoense, M. scrofulaceum, M. kansasii.

M. kansasii
- Most pathogenic nontubercular mycobacteria.
- 2nd **MC** cause of lung disease due to NTM.
- Risk factors: COPD; Silicosis, Lung carcinoma, Prior tuberculosis.
- Clinical features and treatment is similar to tuberculosis with Rifampicin being the most effective drug.

24. Ans. (b) Lymph node *Ref. Dashore Manual of Skin Disease, p 85*

Scrofuloderma is a type of cutaneous tuberculosis.
- It results from direct extension of infections from underlying tuberculous focus, i.e. infected lymph glands, muscles or bones.
- Patient's immunity is poor or moderate.
- Lab investigations – demonstration of bacilli in smears of biopsy material, culture.

25. Ans. (c) Causes decreased efficacy of BCG due to cross immunity *Ref. Park 21/e, p 177; 22/e p 179*

Exposure to some non-tuberculous environmental mycobacteria (M. vacuae, M. non-chromogenicum) may have conferred partial immunity on the population and thus masked the potential benefit of BCG vaccination. There is also evidence that exposure to other species (M. kansasii, M. scrofulososum) have an antagonistic action against BCG.

MOTT (Mycobacteria Other Than Tuberculosis)
- MOTT are mycobacterial species that may cause human disease but do not cause tuberculosis.
- **Spread:** – Unlike tuberculosis, which is spread from person to person, MOTT infections are not considered contagious. There is no evidence that the infection can be transmitted from one person to another. The mode of infection with MOTT is not clear.
- **Clinical features:** – Like tuberculosis, an MOTT infection primarily affects the lungs and the symptoms are similar. Usually MOTT infections progress slowly.
  - *Symptoms include:* Fever, Weight loss, Cough, Loss of appetite, Night sweats, Blood in the sputum.
- **Diagnosis:** MOTT infections can be more difficult to diagnose than tuberculosis. A diagnosis is generally based on the following:
  - Medical history including above symptoms
  - Chest X-ray
  - *Sputum culture:* Several sputum cultures are often necessary.
  - *Other procedures:* More complicated diagnostic procedures (BAL) may be required in certain cases.
- ***Treatment:*** Many MOTT infections are benign with no need for treatment. MOTT infections are naturally resistant to conventional antibiotics and it is necessary to use several ATT at the same time in order to overcome drug resistance.

26. **Ans. (a) *Mycobacterium gordonae*** *Ref. Greenwood 16/e, p 216*

Scotochromogens:
M. Szulgai
M. Scrofulaceum
M. Gordonae/M. acquae
Mnemonic: *Sundar Sushil Girl*

27. **Ans. (a and c) *M. kansasii, M. marinum*** *Ref. Ananthanarayan 8/e 359, 9/e 359*

Photochromogens
M. Siniae
M. Marinum
M. Asiaticum
M. Kansasii

Mnenomic: *Sridevi Marry Anil Kapoor*

28. **Ans. (a) M. Kansasii** *Ref. Ananthanarayan 8/e 359, 9/e 360*

M. kansasii is a slow growing atypical mycobacteria belonging to photochromogens.

Other options

- M. fortuitum and M. Chelonai belong to Group IV of atypical mycobacteria (rapid growers) and their colony usually appear within seven days.
- ***Mycobacterium abscess*** is a rapidly growing mycobacterium that is a common water contaminant. It can cause chronic lung disease, post-traumatic wound infections, and disseminated cutaneous diseases, mostly in patients with suppressed immune systems.

Note:
- *M. abscessus* and *M. chelonae* can be distinguished from *M. fortuitum* by their failure to reduce nitrate and to take up iron.
- Tolerance to 5% NaCl in Löwenstein-Jensen media, and non-utilisation of citrate as a sole carbon source are characteristics that distinguish *M. abscessus from M. chelonae*.

29. **Ans. (a) M. Kansasii** *Ref. Ananthanarayan 9/e, p 360; Harrison 18/e, p 1369; 19/e, p 1129*

As mentioned above M Kansasii is not a rapid grower

Rapid Growing Mycobacteria

- Heterogeneous group to mycobacteria capable of rapid growth, colonies appearing within seven days of incubation.

Important members include:

- M. phlei : Saprophyte
- M. fortuitum : Chronic abscess, pulmonary lesions
- M. chelonae : Chronic abscess
- M. smegmatis : Round in smegma
- M. vaccae : Immunomodulator that stimulates protectine immune response in tuberculosis
- M. abscessus : Oesophageal motility disorders such as achlasia

Chapter Review

1. **Mycobacteria is discovered by:** **[RJ 2006]**
   a. Robert Koch b. Alexander
   c. Vircho d. Callematte
   [Ref. Ananthanarayan 8/e, p 346, 9/e, p 345]

2. **Lepra bacilli can survive outside the human body up to:**
   a. 7 days b. 12 days **[AP 2006]**
   c. Zero days d. 5 days
   [Ref. Ananthnaraya 8/e, p 365, 9/e, p 365]

Lepra bacilli can survive in warm humid environment for 9-16 days and in moist soil for 46 days. They survive exposure to direct sunlight for 2 hours and UV light for 30 minutes.

3. **Tuberculin test is reaction of:** **[UP 2006]**
   a. Anaphyxis mediated b. Cell mediated
   c. Antibody mediated d. Immuno complex mediated
   [Ref. Ananthanarayan 8/e, p 356, 9/e, p 354]

4. **Mycobacteria can be diagnosed on microscopy when counts are:** **[Delhi 2008]**
   a. 10,000 or more/ml b. 1,00,000 or more/ml
   c. 1,000 or more/ml d. 10 or more/ml
   [Ref. Ananthnaraya 8/e, p 352, 9/e, p 352]

5. **H_2SO_4 concentration to stain M. leprae is:** **[Delhi 2006]**
   a. 1% b. 5%
   c. 10% d. 20%
   [Ref. Ananthanarayan 8/e, p 369, 9/e, p 364]

Because M. leprae is less acid fast than M. tuberculosis

6. **BCG vaccine in HIV (+) newborn is:** **[RJ 2006]**
   a. Contraindicated b. Double dilution
   c. Half dilution d. Dose double
   [Ref. Ananthanarayan 8/e, p 357, 9/e, p 356]

BCG vaccine should not be given to infants and children with active HIV disease. Babies born to mother with AFB positive spectum should bot be given BCG at birth, but only after a course of preventive chemotherapy.

7. **Cutaneous lesions may be produced by the following mycobacteria except:** **[Delhi 2001]**
   a. *M. intracellulare* b. *M. leprae*
   c. *M. marinum* d. *M. tuberculosis*
   e. *M. ulcerans* *[Ref. Ananthanarayan 8/e, p 361, 9/e, p 359]*

Mycobacterium causing skin lesion:
- *M. tuberculosis*
- *M. leprae*
- *M. ulcerans*
- *M. marinum*
- *M. haemophilum*

8. **Acid fastness of *tubercle bacilli* is attributed to:**
   a. Presence of mycolic acid **[AIIMS 91, 85; PGI 85]**
   b. Integrity of cellwall
   c. Both of the above
   d. None of the above
   [Ref. Ananthanarayan 8/e, p 348, 9/e, p 346]

9. **In which type of Cutaneous Tuberculosis, caseation is most commonly seen:** **[PGI 93]**
   a. Papulonecrotic
   b. Scrofuloderma
   c. Lupus vulgaris
   d. Erythema nodosum
   [Ref. Greenwood 18/e, p 213]

10. **Single skin lesion is seen in which type of leprosy:**
    a. LL b. TT **[AI 93]**
    c. BL d. BT
    [Ref. Harrison 18/e, p 1362]

11. **Lepra bacilli are best cultivated in:** **[AI 93]**
    a. Guinea pig
    b. Armadillos
    c. Mouse foot pad
    d. Nutrient agar
    [Ref. Ananthanarayan 8/e, p 365, 9/e, p 365]

12. **Which of the following is not a pathogenic mycobacteria?** **[AI 91]**
    a. *M. kansasii* b. *M. scrofulaceum*
    c. *M. cheoleni* d. *M. smegmatis*
    [Ref. Ananthanarayan 8/e, p 347, 9/e, p 361]

Mycobacterium smegmatis is a saprophytic mycobacteria isolated from smegma (though not regularly found in smegma). It is a frequent isolate from soft tissues lesion following trauma or surgery

Other saprophytic mycobacteria include:
- *M. butyricum* from butter
- *M. phlei* from grass
- *M. stercoris* from dung

Answers 1. a. Robert Koch 2. b. 12 days 3. b. Cell mediated 4. a. 10,000 or ... 5. b. 5%
6. a. Contraindicated 7. a. *M. intra...* 8. c. Both of the ... 9. b. Scrofuloderma 10. b. TT
11. b. Armadillos 12. d. *M. smegmatis*

13. *Mycobacterium tuberculosis* is differentiated from other atypical mycobacteria by: [SGPGI 95; AIIMS 91]
a. Niacin test b. AFB staining
c. PAS staining d. None
[Ref. Ananthanarayan 8/e, p 349, 9/e, p 347]

Niacin test is positive in *M. tuberculosis, M. simiae* and few strains of *M. cheloneli.*

14. The local lesion in BCG is maximum in: [Kerala 98]
a. 2 days b. 7 days
c. 28 days d. 1 year
[Ref. Park, 22/e, p 179]

15. Mitsuda reaction is read after: [MP 98]
a. 3 days b. 3 hours
c. 3 weeks d. 3 months
[Ref. Ananthanarayan 8/e, p 368, 9/e, p 367]

16. The factor which promotes, virulence of *M. tuberculosis:*
a. Wax D
b. Cord factor [TN 98]
c. Muramyl dipeptide
d. Mycolic acid *[Ref. Jawetz, 27/e, p 312]*

17. BCG differs from Mantoux test by: [UP 98]
a. Less sensitive and more specific
b. More sensitive and more specific
c. More sensitive and more specific
d. Less sensitive and more specific
[Ref. Ghai 6/e, p 235, 192]

18. Not a pathogenic mycobacterium to human: [TN 99]
a. *M. paratuberculosis* b. *M. kansasii*
c. *M. ulcerans* d. *M. intracellular*
[Ref. Ananthanarayan 8/e, p 360]

19. Rapid diagnosis of tuberculosis is possible with: [ICS 2K]
a. Auramine rhodamine~stain
b. Zeil-Neelsen stain
c. Geimsa stain
d. Leishman stain
[Ref. Ananthanarayan 8/e, p 353, 9/e, p 353]

20. *Mycobacterium scrofulaceum* which cause scrofula, belongs to which groups of atypical mycobacteria: [Kerala 2K]
a. Photochromogens
b. Scotochromogens
c. Non-photochromogens
d. Rapid growers
e. None of the above
[Ref. Ananthanarayan 8/e, p 360, 9/e, p 359]

21. Photochromogenic strain of mycobacterium species is: [Kar 2001]
a. *M. kansasii* b. *M. scrofulaceum*
c. *M. avium intracellular/lare* d. *M. smegmatis*
[Ref. Ananthanarayan 8/e, p 359, 9/e, p 359]

22. Mantoux test is an indicator of: [Kolkata 02]
a. Immediate of hypersensitivity
b. Delayed hypersensitivity
c. Cell-mediated cytotoxicity
d. Of no interest
[Ref. Ananthanarayan 8/e, p 356, 9/e, p 354]

23. The best diagnostic procedure of M. tuberculosis:
a. PCR [SGPGI 02]
b. Auramine rhodamine stain
c. Sputum culture *[Ref. Harrison, 19/e, p 1350]*
d. Sputum examination

24. Drug resistance in tuberculosis is due to: [Kar 2002]
a. Transformation b. Transduction
c. Conjugation d. Mutation
[Ref. Jawetz 27/e, p 316]

25. Subtype of leprosy has maximum number of TH:1 cells: [UP 03]
a. TT b. BB
c. LL d. Borderline leprosy
[Ref. Harrison, 18/e, p 1362]

26. All are rapid growers except: [SGPGI 03]
a. *M. fotuitum* b. *M. chelonei*
c. *M. avium intracellulare* d. *M. smegmatis*
[Ref. Ananthanarayan 8/e, p 361, 9/e, p 359]

27. All of the following are acid fast bacteria except:
a. Cryptosporidia b. Mycoplasma [SGPGI 03]
c. Mycobacteria d. Nocardia
[Learn the list from our book]

28. In a patient, the lymphnodes show necrosis with poor granuloma formation with plentiy of acid fast bacilli sugessts: [SGPGI 04]
a. Tuberculosis in an immunocompromised patient
b. HIV with tuberculosis
c. Sarcoidosis *[Ref. Harrison, 18/e, p 1346]*
d. *Mycobacterium bovis* infection

In HIV positive individuals lymph node TB biopsy reveals poorly organized or no granulomas at all. However bacterial load is heavier than HIV negative individual.

29. *Mycobacterium* non-pathogenic to man:
a. *M. bovis* b *M. kansai* [Kolkata 05]
c. *M. avium intracellular* d. *M. phlei*
[Ref. Ananthanarayan 8/e, p 360, 9/e p 361]

M. phlei is a saprophyte

Answers 13. a. Niacin ... 14. c. 28 days 15. c. 3 weeks 16. b. Cord fa ... 17. b. More sensitive
18. a. *M. paratuber* ... 19. a. Auramine 20. b. Scotoch ... 21. a. *M. kansasii* 22. b. Delayed ..
23. a. PCR 24. d. Mutation 25. a. TT 26. c. *M. avium* ... 27. b. *Mycoplasma*
28. a and b Tubercudo...and HIV... 29. d. *M. phlei*

30. Swimming pool granuloma is caused by: [UP 05]
a. *M. kansasi* b. *M. avium intracellulare*
c. *M. scrofulaceum* d. *M. marinum*
[Ref. Ananthanarayan 8/e, p 361, 9/e, p 360]

31. Most common organism in AIDS in TB, India:
a. *M. tuberculosis* [MP 06]
b. *M. avium intracellulare*
c. *M. bovis*
d. *M. ulcerans* *[Ref. Ananthanarayan 9/e, p 576]*

32. Stain for the diagnosis of tuberculosis: [MP 07]
a. Auramine-rhodamine
b. India ink
c. Geimsa stain
d. Zeil-Neelsen stain
[Ref. Ananthanarayan 8/e, p 353, 9/e, p 353]

33. Globi is: [Jharkhand 05]
a. Histiocyte containing acid-fast bacillus
b. Lymphocyte containing acid-fast bacillus
c. Nutrophill containing acid-fast bacillus
d. Large lymphocyte containing acid-fast bacillus
[Ref. Ananthanarayan 8/e, p 365, 9/e, p 364]

34. BCG, True about it: [UP 07]
a. Killed vaccine
b. Subcutaneosly given
c. Given in positive tubercular patients
d. Live vaccine
[Ref. Ananthanarayan 8/e, p 356, 9/e, p 355]

35. Which of the following mycobacteria can cause disease in HIV +ve patient with a CD4 count of 600/cu.mm: [Comed 07]
a. *M. tuberculosis* b. MAC
c. *M. chelonei* d. *M. fortuitum*
[Ref. Harrison 18/e, p 1548]

- Patients with HIV infection are more likely to have active TB by a factor of 100.
- Median CD4 + T cell count at presention of TB was 326/µl
- MAC infections are seen when CD4 count falls below 50 µl

36. Rapid growing mycobacterium bacilli pathogenic to humans are: [Manipal 08]
a. *M. chelonei* b. *M. marinum*
c. *M. avium* d. *M. ulcerans*
[Ref. Ananthnaraya 8/e, p 360, 9/e, p 361]

37. Generation time of lepra bacilli is: [Manipal 06]
a. 12 days b. 5 minutes
c. 10 hours d. 24 hours
[Ref. Ananthanarayan 8/e, p 365, 9/e, p 365]

38. The medium used for *Mycobacterium tuberculosis* is:
a. Sabouraud's medium [AI 96]
b. LJ medium
c. Pick's medium
d. NIH medium
[Ref. Ananthanarayan 8/e, p 348, 9/e, p 346-347]

39. Rapid examination of Tubercle bacilli is possible with:
a. Ziel-neelsen stain [AI 96]
b. Kinyoun stain
c. Auramine-Rhodamine stain
d. Giemsa stain
[Ref. Ananthanarayan 8/e, p 353, 9/e, p 353]

- ***Fluorescent microscopy*** is preferred when several smears are to be examined daily ***(rapid screening).***
- In this, smears are stained with auramine phenol or auramine rhodamine fluorescent dyes and examined under UV illumination.
- Bacilli will appear as bright rods against dark back-gound.

40. In TB, immunity is provided by: [PGI 98]
a. CD4+
b. CD8+
c. IgG
d. IgM
[Ref. Ananthanarayan 8/e, p 351, 9/e, p 350]

- Only specific immune mechanism effecitve in TB is the *'cell mediated type'* ***(since it is intracellulare).***
- Humoral immunity appears to be irrevalent.
- ***Key cell*** is the activated **CD4+ Helper** T cell.

Answers **30.** d. *M. marinum* **31.** a. *M. tuberculosis* **32.** a. Auramine ... **33.** a. Histiocyte ... **34.** d. Live vaccine
35. a. *M. tuberculosis* **36.** a. *M. chelonei* **37.** a. 12 days **38.** b. LJ medium **39.** c. Auramine...
40. a. CD4+

NEET Pattern Questions

1. Which of the following is photochromogenic:
a. M. kansasii b. M. scrofulorum
c. M. intracellulare d. M. avium
[Ref. Ananthanarayan, 8/e, p 359, 9/e, p 359]

2. Which of the following is not a pathogenic mycobacteria:
a. M. kansasii b. M. scrofulorum
c. M. cheolonei d. M. smegmatis
[Ref. Ananthanarayan, 9/e, p 361]

3. Buruli ulcer is caused by:
a. Streptococcus b. Spirillium minus
c. M. ulcerans d. Brucella
[Ref. Ananthanarayan, 8/e, p 361, 9/e, p 361]

4. Tuberculosis complex include all except:
a. M. tuberculosis b. M. bovis
c. M. kanasasii d. M. microti
[Ref. Ananthanarayan, 8/e, p 365]

5. Fish tank granuloma is seen in:
a. M fortuitum
b. M kanasasi
c. M marinum
d. M leprosy *[Ref. Ananthanarayan, 9/e, p 362]*

Fish tank granuloma (= swimming pool granuloma) is caused by M. marinum, a natural pathogen of cold blooded animals. Normally it lives as saprophyte in salt and fresh water. Lesion begin as papule which break down to form an indolent ulcer. Usually occurs at body prominences like elbow, knee, ankle, nose, fingers or toes.
Ulcer are self limiting and undergo spontaneous healing.

6. Pigment producing atypical mycobacteria:
a. M. fortuitum and M. chelonae
b. M. xenopi and MAC
c. M. gordonae and M. szulgai
d. M. ulcerans

M. scheelgai and M. gordonae are scotophoto chromogen, which produce pigment in dark

7. Reactive tubercular arthritis:
a. Spina ventusa
b. Pott's disease
c. Poncet's disease
d. None

8. ENL is seen in:
a. Lepromatous leprosy
b. Tuberculoid leprosy
c. Indeterminate leprosy
d. Pure neuritic leprosy *[Ref. Harrison, 18/e, p 1362]*

9. Liquid medium for tuberculosis:
a. LJ medium
b. Dorset medium
c. Loeffler's medium
d. MGIT *[Ref. Jawetz, 27/e, p 308]*

Culture media for M.tuberculosis
- Semisynthetic agar media: Middle brook 7H10 and 7H11 contains salt, vitamins, albumin, catalase and glycerol. Requires large inocula which makes them less sensitive for primary isolation of mycobacteria.
- Inspissated egg media: Lowenstein Jensen media, small inocula can be grown on these media in 3-6 weeks. These media with antibiotics (gruft and mycobactosel) are used as selective media.
- Broth media: Middle brook 7H9 and 7H12. Support small inocula. Eg MGIT system, versa TREK culture system and MB redox are other examples.
- Use of liquid media with radiometric growth detection such as BACTEC 460 has simplified culture methods and enabled results to be given in 2-3 weeks.

10. Live TB bacilli culture is by:
a. Tinsdale medium
b. MGIT
c. MYPA medium
d. BYCE agar *[Ref. Jawetz, 27/e, p 309]*

11. Radiomimetric BACTEC detect growth of M tuberculosis in how much time:
a. 1 week b. 2-3 week
c. 4-8 week d. >10 weeks
[Ref. Ananthanarayan, 9/e, p 354]

Answers 1. a. M. kansasii 2. d. M. smegmatis 3. c. M. ulcerans 4. c. M. kansasii
5. c. M marinum 6. c. M. gordonae 7. c. Poncet's disease 8. a. Lepromatous
9. d. MGIT 10. b. MGIT 11. b. 2-3 weeks

12. Mycobacterium tuberculosis grows in LJ media in:
a. 10-14 days
b. 2-3 weeks
c. 4-8 weeks
d. >10 weeks *[Ref. Jawetz, 27/e, p 309]*

13. Which type of pulmonary TB is most likely to given sputum positive:
a. Fibronodular
b. Pleural effusion
c. Cavitary
d. None *[Ref. Ananthanarayan, 9/e, p 351]*

14. Fastest method for diagnosis of TB:
a. Gene expert
b. LJ medium
c. TB MGIT
d. BACTEC *[Ref. Jawetz, 27/e, p 315]*

Genexpert MTB/RIF test, a real multiplex PCR method that both identifies the MTB complex and also detects genes that encode rifampin resistance within 2 hours. Overall sensitivity is 98.2%/for smear positive cases and 72.5% for smear negative samples. Overall specificity is 99.2%.

15. Tuberculin test is:
a. Intramuscular
b. Intradermal
c. Subcutaneous
d. None *[Ref. Ananthanarayan, 9/e, p 355]*

16. Method used for acid fast staining:
a. Robertson's method
b. Ziehl-Neelsen
c. Silver impregnation method
d. Dark ground illumination
[Ref. Ananthanarayan, 9/e, p 346]

17. Acid fastness of tubercle bacilli is attributed to:
a. Presence of mycolic acid
b. Integrity of cell wall
c. Both of the above
d. None of the above *[Ref. Ananthanarayan, 9/e, p 346]*

Acid fastness is attributed to presence of mycolic acid. It is related to integrity of cell wall and appears to be the property of lipid rich waxy cell wall.

18. XDR-TB is defined as Resistance to:
a. INH plus rifampicin
b. Fluoroquinolones plus INH plus amikacin
c. Fluoroquinolones plus rifampicin plus kanamycin
d. Fluoroquinolones plus INH plus rifampicin plus amikacin *[Ref. Greenwood, 18/e, p 219]*

19. Positive tuberculin test means:
a. Resistance to TB
b. Susceptibility to TB
c. Hypersensitivity
d. None of the above *[Ref. Park, 22/e, p 172]*

Positive reaction means person is infected with M. tuberculosis and does not necessarily means person is suffering from disease

20. To notify a slide as AFB negative minimum how many fields should be checked?
a. 20 b. 100
c. 50 d. 200
[Ref. Park, 22/e, p 170]

21. Modified Ziehl-neelsen staining is used for:
a. Mycobacterium tuberculosis
b. Mycobacterium bovis
c. Nocardia
d. All of the above *[Ref. Ananthanarayan, 9/e, p 346]*

Ziehl-Neelsen Stain
- Described by F. Ziehl and F. Neelsen.
- Used to identify acid fast organism.
- Reagents used are carbol fuchsin, acid alcohol and methylene blue.
- *Technique*: The slide is first stained with carbol fuchsin which is then heated to dry and rinsed off in tap water. The slide is then flooded with a 1% solution of hypochloric acid in ispropyl alcohol. There after the slide is stained with methylene blue.

Modifications:
- 1% sulfuric acid alcohol: Nocardia, Actinomycetes 0.5%
- 0.5% Sulfuric acid alcohol: Oocysts of isospora, coyclospora cryptosporidium
- 0.25%–0.5% sulfuric acid alcohol for bacterial endospores.
- *Kinyoun modification* (does not require heat): Nocardia, mycobacteria

22. MDR TB is defined as:
a. Resistance to INH and Ethambutol
b. Resistance to Rifampicin and Ethambutol
c. Resistance to Pyrazinamide and Rifampicin
d. Resistance to INH and Rifampicin
[Ref. Greenwood, 18/e, p 219]

Answers 12. c. 4-8 weeks 13. c. Cavitary 14. a. Gene expert 15. b. Intradermal 16. b. Ziehl-Neelsen
17. c. Both of the above 18. d. Fluoroquinolones plus INH ... 19. d. None of the above
20. b. 100 21. d. All of the above 22. d. Resistance to INH and Rifampicin

23. Gne expert used for getting diagnosis of TB in:
a. 1–2 hrs
b. 5 hrs
c. 10 hrs
d. 20 hrs *[Ref. Jawetz, 27/e, p 315]*

24. Hansen's bacillus is cultured in:
a. L J medium
b. Robertson's cooked meat medium
c. Foot pad of mice
d. Sabraud's agar *[Ref. Ananthanarayan, 9/e, p 365]*

25. True about mycobacterium leprae:
a. Transmitted by droplet infection
b. Phenolic glycolipid (PGL) is virulence factor
c. Generation time 12–13 days
d. All are true *[Ref. Ananthanarayan, 9/e, p 365]*

26. Globi in leprosy consist of:
a. AFB + macrophages
b. Neutrophils + AFB
c. Platelet plug
d. None of the above

[Ref. Park, 22/e, p 172]

In lepromatous leprosy, the dermis is filled with rounded macrophages stuffed with bacilli which often form clumps termed Globi.

27. Primary complex of M bovis involves:
a. Tonsil and lung
b. Tonsil and intestine
c. Tonsil and skin
d. Skin and Intestine *[Ref. Greenwood, 18/e, p 213]*

28. Phagocytosis of mycobacterium tuberculosis by macrophages is mainly mediated by:
a. IL 6
b. IL 3
c. IL 12
d. IFN Gamma

[Ref. Greenwood, 18/e, p 214]

29. Battey bacillus is:
a. Klebsiella pneumoniae
b. Mycobacteria paratuberculosis
c. Klebsiella ozaenae
d. Mycobacteria intracellulare

[Ref. Ananthanarayan, 9/e, p 360]

M. introcellulare is commonly known as Battey bacillus becused it was first identified as a human pathogen at Battey state hospital USA.

30. Organism identified by interferons:
a. Staphylococcus
b. Leptospira
c. Campylobactor
d. M. tuberculosis *[Ref. Harrison, 19/e, p 1115]*

Answers **23.** a. 1–2 hrs **24.** c. Foot pad of mice **25.** d. All are true **26.** a. AFB + macrophages
27. b. Tonsil... **28.** d. IFN gamma **29.** d. Mycobacteria intracellulare **30.** d. M. tuberculosis

CHAPTER

15

Enterobacteriaceae

- **Enterobacteriaceae** includes large group of gram-negative rods whose natural habitat is large intestine.
- They are predominantly aerobic or facultative anaerobes, non-sporing and non-acid fast.
- They have following **common characteristics:**
  - Grow well on ***MacConkey* media.**
  - Catalase (+)ve; except *Shigella dysentriae* type 1.
  - Oxidase (-)ve; reduces nitraxtes to nitrite.
  - Urease (-)ve; except *Proteus, Klebsiella, Morganella.*
  - Motile by peritrichate flagella except *Shigella, Klebsiella* and *Salmonella gallinarum-pullorum.*
  - Ferment glucose except *Shigella.*

I

- Gram (-)ve bacilli
- Catalase positive
- Oxidase negative
- Classified on the basis of lactose fermentation.

Classification

- *MC* and oldest method to classify these bacteria is on ***basis*** of ***fermentation of lactose.***

Lactose fermented rapidly = Coliforms	Lactose fermented slowly = Late lactose fermenter = Paracolons	Lactose not fermented = Mostly pathogenic
• *Escherichia* • *Klebsiella* • *Enterobacter aerogenes* They forms pink colony on Maconkey's medium and are usually part of normal flora.	• *Edwardsiellla* • *Serratia* • *Citrobacter* • *Arizona* • *Providencia* • *Erwinia* • *Shigella sonnei*	• *Shigella except S. sonnei* • *Salmonella* • *Proteus*

Remember: Differential media which distinguishes lactose fermenting (colored) from non-lactose fermenting colonies (non pigmented) includes eosin methylene blue (EMB), MacConkey or deoxycholate medium.

Escherichia coli

Morphology

Motile by peritrichate *flagella,* non-sporing. Many pathogenic isolates are *capsulated.*

Culture and Biochemical characteristics

- Grows well on ordinary media.
- *On blood agar shows hemolysis.*
- Indole and Methyl red (+)ve while VP and citrate (-)ve [IMVC ++--].
- Except certain verocytotoxin producing strain *E. Coli* are lactose fermentor.

Antigenic structure

- *Somatic antigen O* – Lipopolysaccharide in nature, heat stable. Antibodies to O-antigen are predominantly IgM.
- *Capsular antigen K* – Heat labile responsible for attachments of bacteria to epithelial cells.
  – Associated with virulence.
- *Flagellar antigen*
- Serotyping based on these three antigens is:
  - **Normal colon strains = commensal strains belong to early 'O' group (1, 2, 3, 4, etc.).** These strains generally lack specialized virulent traits. However, they may be involved in extraintestinal infection.
  - **Enteropathogenic strains: Belong to later 'O' group (25, 55, 86 etc).**

I

E. coli toxins
- Heat stable toxin: Rapid action.
- Heat labile toxin: Activate cAMP.
- Verocytotoxin: Phage coded inhibit protein synthesis

- **Extraintestinal pathogenic *E. coli* (EXPEC):** *MC* cause of extraintestinal *E. coli* infection. Like commensal *E.coli* they can be found in normal intestinal flora without causing gastroenteritis, but they have acquired diverse virulence factor that enable them to live extraintestinally.

Virulence factors

a. Surface antigen:

- O-antigen – It has endotoxic activities and also protects bacteria from phagocytosis. These are lipopolysaccharide and are heat stable.
- K-antigen – Protect against phagocytosis.
- Fimbriae – *Plasmid coated fimbriae increase virulence*, while chromosomally determined fimbriae has no effect on virulence.
- H-antigen – Heat labile and group specific antigen. Occurs in single phase except in Salmonella where it biphasic.

b. Toxin: *E.coli* produce two kind of exotoxin:
- Hemolysins – No role in pathogenesis of dirrhea.
- Enterotoxins – Important in pathogenesis of diarrhea.

E. coli enterotoxins		
Heat stable toxin (STA)	**Heat labile toxin (LT)**	**Verocytotoxin (VT) = Shiga like toxin (SLT)**
• Plasmid mediated • Activates cGMP • Acts very rapidly	• Plasmid mediated • Consist of 2 subunit A → Activates CAMP → Diarrhea B → Binds GM_1 gangliosides	• Phage coded • Belong to ribosome inactivating protein [RIP] toxins • A subunit of SLT inhibit ribosome and ↓ protein synthesis and shows cytotoxicity

Clinical Findings

A. Diarrhea – 5 types of diarrheogenic *E. coli* are recognized:
- **Enteropathogenic = enteroadherant *E. coli***
  - Cause diarrhea in infant and children.
  - Strains that carry PEAF plasmid are termed typical EPEC while those lacking it are called atypical …*Greenwood 18/e, p283.*
  - Non-toxigenic and non-invasive.
  - Produce diarrhea by disruption of brush border. Bacilli adhere to upper small intestine through adhesion receptor synthesized by bacteria which is then inserted in to host put wall
  - They are unable to ferment sorbitol.
  - Usually cause epidemic, but sporadic cases can also occur.
- **Enterotoxigenic *E. coli* (e.g. 06, 08, 015, 025, 027, 0167)**
  - Affect *all age group.*
  - *MC* cause of traveller's diarrhea (indistinguishable from cholera). Most cases are endemic.
  - Produce either or both LT and STa.
  - Produce diarrhea, only when it adheres to intestinal mucosa by fimbrial or colonization factor antigen and produce toxin.
- **Enteroinvasive *E. coli***
  - Called *atypical E. coli* as many strains do not ferment lactose or ferment it late.
  - Resemble *Shigella flexneri* except in fermenting dulcitol and forming alkali in litmus milk.
  - Cause illness resembling *shigellosis.*
  - Ability to *penetrate cells is due to* presence of plasmid which codes for outer membrane antigen called virulence marker antigen *[VMA].*
  - For *diagnosis* of EIEC - Sereny test is used. Cell penetration of Hela or HEP-2 in culture is also *diagnostic test.*

- **Enterohemorrhagic *E. coli* (EHEC) = Verotoxingenic *E. coli* (VTEC)**
  - Source of infection appears to be salad as washing only doesn't remove bacteria.
  - Produce *Verocytotoxin VT* whose major target is vascular endothelial cells.
  - VT resembles shiga toxin, genes encoding VT in E. coli are carried on a lambda like bacteriophage (in shigella they are present on chromosome). It acts by inhibiting protein synthesis.
  - Cause disease ranging from mild diarrhea to fatal hemorrhagic colitis and hemolytic uremic syndrome (HUS).
  - **HUS:** - Mainly caused by O157 H7 type which does not ferment sorbitol (Some 0157 and non 0157 strain ferment sorbitol).
    - Sorbital Mac conkey medium helps in screening of 0:157VTEC
    ..... *Ananthanarayan 9/e, p 279*
    - Antibiotics increase the incidence of HUS.
- **Enteroaggregative E. coli (EAEC)**
  - Cause *persistent diarrhea.*
  - Stacked brick' formation on Hep -2 cells.
  - Produce *EAST* – Enteroaggregative heat stable enterotoxin.

I

Enteroadherant E. coli: Infant and Children

ETEC: All age group.

EIEC: Resemble *Shigella*

EHEC: Produce verocytoxin, causes hemorrhagic colitis and hemolytic uremic syndrome.

Enteroaggregative *E. coli:* Persistant diarrhea

I

EHEC is the commonest cause of hemolytic uremic syndrome

– Usually affects children < 5 years of age

B. UTI

- ***MC cause of both uncomplicated and nosocomial UTI.***
- UTI causing serotypes are those normally found in feces.
- Only one serotype is generally isolated from infected urine at a time while in diarrhea many serotypes are present in a single culture.
- Nephropathogenic *E. coli* typically produce Hemolysin.
- Pyelonephritis is associated with specific type of pilus, P. pilus.
- ***Diagnosis***
  - UTI is said when there is: - Bacteriuria $\geq 10^5$/ml in asymptomatic
    - Bacteriuria of $\geq 10^4$/ml in symptomatic
    - Bacteriuria of $\geq 10^2$/ml in catheterized sample
    - Bacteriuria of any degree in suprapubic aspirate.
- **Presumptive diagnosis of bacteriuria is made by:** - *Griess nitrite test*
  - *Catalase nitrite test*
  - *Triphenyl tetrazolium chloride test.*

C. **Pyogenic Infection** – *E. coli* is ***MC*** Cause of intrabdominal abscess, peritonitis and cholangitis.

D. **Meningitis** – *E. coli* is ***MC*** cause of neonatal meningitis. 75% of miningitis causing E. coli have the K1 antigen. This antigen crossreacts with the group B capsular polysaccharide of N. Meningitidis.

E. **Pneumonia** – *E. coli* is **2nd *MC*** cause of nosocomial pnenumonia (*1st being* ***Staph. aureus***)

F. **Bacteremia** – UTI is ***MC*** source of *E. coli* bacteremia leading to septic shock

I

E. coli is the MC cause of:

– UTI

– Neonatal meningitis

– Intra-abdominal abscess

Treatment

- **UTI – *Fluoroquinolone* are *DOC.***
- **Diarrhea – Fluids and electrolyte correction with *no antibiotics.***
- **Drug resistance is plasmid mediated.**

Klebsiella

- Non motile, capsulated (capsule seen as haloes around bacilli), gas producing rod.
- **Classified** into **3 species** on the basis of **biochemical characteristics** and into **serotypes** on *basis* of ***K-antigen*** (*capsular antigen*).

I

Capsule of type II resemble that of pneumococcus. Polysaccharide capsule is the major virulence factor

Klebsiella pneumoniae = Friedlander's bacillus

- Rarely cause disease in normal person.

Clinical manifestation

- Cause community acquired pneumonia in alcoholics, chronic bronchopulmonary disease or diabetes.

- *MC* clinical syndromes it causes are pneumonia, UTI, abdominal infections, surgical site infection, soft tissue infection and bacteremia.
- **Pneumonia :**
  - Mainly hospital acquired. Abscess are more common than in pneumococcal pneumonia.
  - Cause classic lobar infiltrate with bulging fissure.
  - Sputum is red current jelly in character.
- **UTI:** Mainly in settings of prolonged catheterization.
- **Diarrhea:** By toxin similar to ST_A of *E. coli*.
- Virulence is increased in iron overload conditions like thalassemia.

I

Klebsiellae: Non-motile, capsulated Gram (–)ve
- *K. pneumoniae:* Pneumonia, UTI
  - One of the common cause of neonatal sepsis in India
- *K. ozaenae:* Ozoene
- *K. rhinoscleromatis* *Rhinoscleromais*

Klebsiella ozaenae

- Causative agent of ozoena characterized by foul smelling nasal discharge.

Klebsiella rhinoscleromatis (Frisch bacillus)

- Causative agent of rhinoscleroma (Hebra nose).

Treatment of Klebsiella

- **Carbipenim** (Imipenam) – Most active antibiotic against *klebsiella*.

Shigella

- Non motile, non capsulated, non lactose fermentar (except *S. sonnei* which ferments it late).
- **Classified** on the *basis of somatic O Antigen (LPS) and carbohydrate fermentation (mannitol) pattern.*
- Catalase is produced by *all except Sh. dysnteriae I.*
- Mannitol is fermented by *all except Sh. dysnteriae I.*
- ***MC*** Shigellosis worldwide – *Sh. sonnei.*
- ***MC*** Shigellosis in India – *Sh. flexneri.*
- Most clinically severe form of Shigellosis is caused by *Sh. Dysenteriae* type I while mildest form of bacillary dysentery is by *Sh. sonnei* (may occur as food poisoning).
- Only species that exist as single serotype – *Sh. sonnei.*
- **Pathogenic species of shigella are:**

Species	Group	Ornithine Decarboxylase	Mannitol Fermentation
S. dysenteriae	A	–	–
S. flexneri	B	+	–
S. boydii	C	+	–
S. sonni	D	+	+

Culture

- **Selective media:**
  - Xylose-lysine deoxycholate citrate agar (DCA).
  - Hektoen enteric agar or Salmonella - Shigella agar.

..... *Jawetz 27/e, p 238*

- ***Triple sugar iron*** (TSI) agar is used to differentiate salmonella and Shigella from other gram-negative rods in stool cultures.

I

Selective media for shigella:
– DCA Agar
– Hektoen enteric agar
– Salmonella–Shigella agar

Pathogenesis

a. **Invasiveness (main):**
   - Bacteria invade basolateral surface of colon epithelium → intracellular replication and cell to cell spread with the help of microbial protein Ics A (ATP-ase) and host protein cadherin L - CAM.
   - This process present in all virulent shigellae as well as in EIEC. It is responsible for late dysentery. Hence *nontoxic mutants can cause dysentery but non-invasive can't produce dysentery.*
   - Pathogenic strains carry a plasmid which is thremoregulated; such that strains become invasive when growing at 37°C but not at 30°C.

b. Toxins:

i. *Endotoxin* - LPS present in all shigella causing irritation of bowel.

ii. *Shigella Dysenteriae* - I produces a heat labile exotoxin (*Shiga bacillus exotoxin*) that affect both gut and CNS:

- It has neurotoxicity on blood vessel of CNS and can lead to miningsmus and coma.
  - *Enterotoxicity* causing fluid accumulation in ligated rabbit ileal loop.
  - *Cytotoxicity* same as Verotoxin I or Shiga like toxin produced by some strain of EHEC including 0157:H7.
- *Toxin has two peptide subunit. A unit (N-glycosidase) of cytotoxin* hydrolyzes adenine from specific sites of 60s RNA and thus *inhibits* ***protein synthesis.*** It contributes to fatal nature of *S. dysenteriae* infection.
- Toxins produce early, non bloody voluminous diarrhea.
- Genes encoding shiga toxin are located on chromosome

I

Shigella

- Non motile, non-capsulated.
- Catalase (–)ve except Sh. dysenteriae
- Infective dose of bacillary dystentery is as low as 10 viable bacteria
- Shiga toxin: Chromosome coded toxin, that inhibits protein synthesis

Clinical Features

- Most cases of bacillary dysentery occur in < 10 year children.
- Infective dose is in the order of 10^3 organisms (*while in vibrio and Salmonella 10^6-10^9*).
  ..... *Jawetz 27/e, p 238*
- Transmitted fecorally generally direct person to person contact; *anal-oral sex* (*esp. in Sh. sonnei*).
- Majority of lesion are in *distal colon.*
- **Complications** – Toxic dilatation, Colonic perforation.
  – *Extraintestinal* (esp. with *S. dysenteriae* and *S. flexneri*) like HUS, Thrombotic thrombocytopenic purpura reactive arthritis, Reiter's syndrome.

Diagnosis

- *Specimen* – Best to use mucus flakes of stool.
- *Transport media* – Sach's buffered glycerol saline.
- *Specific diagnosis* – Culture of shigella from stool.
- Culture media – DCA agar, MacConkey agar
- Pale non-lactose fermenting colonies suggest its diagnosis.

I

For Shigella dysenteriae diagnosis faecal specimen is preferred over rectal swab.

Treatment

- **Mild to moderate dehydration** – Oral rehydration therapy (No antibiotics).
- **Severe cases with bloody diarrhea** – *DOC* Ampicilin or co-trimoxazole (Amoxicillin is not effective).
- *DOC* **for multiresistant:** Nalidixic acid.

Salmonella

- Genus consist of bacilli that parasitise small intestine leading to enteric fever, gastroenteritis, septicemia.
- They are motile with peritrichate flagella except *S. gallinarum pullorum.*
- They are non-capsulated and non-sporing.

Culture and Biochemical characteristics

- Grows readily on simple media.
- On Wilson - Blair bismuth sulphite media S. typhi produce jet black colonies due to production of H_2S.
- ***Enrichment media*** - Selenite F and Tetrathionate broth.
- ***Selective media*** – Salmonella-shigella agar, Deoxycholate citrate agar which promotes growth of salmonella over other enterobacteria; xylose-lysine Deoxycholate agar
- Salmonella ferment sugar producing acid and gas except *S. typhi which is anerogenic.*
- *S. typhi* need *tryptophan as growth factor.*
- Boiling or chlorination of water and pasteurization of milk destroy the bacilli.

Note: DCA & XLD agar are usefull selective media to differentiate shigella and salmonella. Shigella donot exhibit black centre whereas salmonella appear red with black centre.

I

Salmonella
- Non capsulated, non-sporing, motile (except S. gallinarum pullorum).
- Enrichment media: Selenite F and tetrathionate broth
- Selective media: SS agar, DCA media

Classification

- Originally Salmonella was classified on the basis of *O, H* and *Vi antigenic* structure and biochemical reactions. But now on the *basis of DNA hybridization* studies only *7 groups have been identified*. Currently, the genus salmonella is divided into two species: Salmonella enterica (which includes five serotypes) and Salmonella bongari. *...Jawetz 27/e p 239*
- ***Nearly all the salmonella serotypes that infect human are in DNA hybridization group 1.***

Method for isolation of salmonellae

- Differential culture Media: Bismuth sulfite medium permits rapid detection of salmonella (forms black colonies because of H_2S production). EMB, Mac conkey or deoxycholate medium are other differential media, but all lactose nonfermenters grow on these media.
- Selective Culture Medium: Shigella-Salmonella agar; Hektoen entire agar; XLD; deoxy cholate - citrate agar. These mediums favors growth of salmonella and shigella over other enterobacteriaceae.
- Enrichment culture: ***Selenite F or tetrathionate broth,*** both of them inhibit replication of bacterias other than salmonella.

Antigenic structure

1. **Flagellar antigen (H)**
   - Heat labile protein which is strongly immunogenic. When mixed with antisera, it rapidly produce-*flufty clumps*. Exhibit the property of diphasic variation.
2. **Somatic antigen (O)** (Biovin antigen)
   - Heat stable phospholipid polysaccharide complex identical with endotoxin. It remains unaffected by boiling. When mixed with antisera, forms chalky, granular clumps.
   - Located in outer membrane, anchored to cell wall, on the basis of difference in long chain LPS, 30 serotypes are defined.
3. **Vi antigen**
   - Heat labile surface polysaccharide antigen enveloping O antigen. Found only in *S.typhi* and *S.paratyphi*. Poorly analog to K antigen of coliforms. Heat-labile and inmmunogenic. *Associated with Virulence*.

Remember: Order of immunogenic nature of antigen: H > O > Vi

Pathogenicity

- On reaching gut, bacilli are phagocytosed.
- Salmonella typhi *resist intracellular killing* by macrophages and polymorphs and enter mesentric lymph node and from there via thoracic duct enter blood stream to produce bacteremia (Enteric fever).
- All the clinical manifestation begin after ileal penetration

Clinical Manifestation

1. **Enteric fever**

Systemic disease characterized by fever and abdominal pain caused by *S.typhi* (called as typhoid) and S. paratyphi. A, B and C.

S. paratyphi A is emerging as the most common cause of enteric fever specially in Asia.

- *I.P.* – 14 days.
- Infection is acquired through ingestion
- Infective dose: 10^3 - 10^8
- Paratyphoid fever of *S. paratyphi* is generally milder.
- *Mode of transmission* – Transmitted through feco-ral; uro-oral route.
  - Making water as major vehicle
- *Typical features*
  - *Step ladder pyrexia with relative bradycardia*
  - *Rose spots* (located primarily on chest) usually appear at the end of 1st week and resolve after 2 - 5 days.

Carriers:

- Those who excrete bacilli for more than a year are called as Chronic carrier.
- Carrier rate is 3% (i.e. 3% of cases become chronic carrier).
- Though cases occur more in males, carriers are common in females.
- Fecal carrier are MC.
- Urinary carrier signify abnormality in urinary tract.

Diagnosis (Mnemonic: BASU)

A. *Cases*

- **Blood culture (B)**
  - Positive in 1st week (*Diagnostic gold standard*). Sensitivity is 40-80%
  - Clot culture has *higher rate of isolation*, than blood culture.
- **Bone marrow culture** : 55-90% sensitive and unlike that of blood culture, its yield is not reduced by prior antibiotic therapy.
- **Agglutination (A)** = Widal reaction
  - Detects antibodies against O and H antigen. Titer > 1:320 against O antigen and >1: 640 agaist H antigen is considered positive.
  - Becomes (+)ve by end of 1st week, *peaks at 3rd week*, decline afterwards.
- ***Stool culture (S)*** – 3rd week, particular valuable in *patient on antibiotics* in which blood culture is (-) ve Sample are plated directly on *MacConkey, DCA*, and *Wilson - Blair media (highly selective)*.
- ***Urine culture (U)*** – (+)ve only in 2nd and 3rd weeks.

B. *Carriers*

- **Widal** – No value in detection of carrier.
- *Demonstration of Vi agglutinin has been claimed to indicate carrier state and used as screening of carrier.*

Treatment

- **Cases:**
  - First line: Ciprofloxacin, Ceftriaxone
  - Alternative: Azithromycin
  - Multidrug resistant [MDR] *S. typhi*: Ciprofloxacin is ***DOC***. Multidrug resistant is due to R factor.
- **Carriers:** Amoxicillin, Co-trimoxazole, Ciprofloxacin are effective.
- **Control of typhod fever**
  - ***Isolation:*** The cases of typhoid should be isolated till three bacteriologically negative stools and urine reports are obtained on three separate days.
  - ***Disinfection of stool and urine:*** Stool and urine should be disinfected with 15% cresol for at least 2 hours, so as to kill all bacteria. All soiled cloth and linen should be soaked in a solution of 2% chlorine and steam-sterilized.
  - **Immunization:** Following vaccines are available:

Vaccine	Nature	Dose	Minimum age
Type 21a	Live attenuated	4 oral dose	6 years
Vicps	Purified Vi polysaccharide	1 Parenteral dose (subcutaneous/IM)	2 years
VirEPA	Vi bound to recombinent protein	2 parenteral dose	6 month

Vir LPA is most effective.

II. **Gastroenteritis** = (Food poisoning)

- *MC* caustive agent is *S. typhimurium.*
- Most frequent sources are poultry, meat, milk and milk products.
- Never caused by *S. typhi.*
- *IP*-6-48 hours.
- ***Clinical features*** – Nausea, vomiting, diarrhea, Abdominal cramps and fever.
- Blood culture is negative.
- ***Treatment*** – No antibiotics are given usually, except for serious invasive cases.

I

- Bone marrow culture is the most reliable method for diagnosis of enteric fever.

  ...Greenwood 18/e, p 270
- S. paratyphi A is now becoming a predominant cause of enteric fever specially in Asia
- Recomblinant vir EPA is the most effective vaccine.

I

Salmonella carrier

- Site: Biliary tract
- Diagnosis: Culture of duodenal aspirate
- IgG Vi antibodies

I

Proteus mirabilis
- Non lactose fermenter
- Produce enzyme phenytalanine deaminase
- Cause UTI, pneumonia.

III. Bacteremia and Endovascular infection

Salmonella serotypes S. cholerae-suis and S. dublin are frequently associated with sustained bacteremia.

IV. Localized infection

- Intra abdominal infection: Hepatic or splenic abscess, cholecystitis.
- CNS infection: NTS meningitis most commonly develops in infants 1-4 months of age. It can cause death in up to 60% of cases.
- Pulmonary infection: Cause pneumonia and associated complications in patients with lung ca, sickle cell disease or glucocorticoid use.
- UTI and genital infection: Risk factors include malignancy, urolithiasis, structural abnormalities, HIV infection, transplant recepient.
- Bone joint and soft tissue infection: Salmonella osteomyelitis most commonly affects femur, tibia, humurus and is most often seen in conjuction with sickle cell disease, hemoglobinopathies, or pre-existing bone disease.

Proteus bacilli

- Non lactose fermenter, highly motile.
- ***P. Mirablis*** which is responsible for 90% of proteus infection is a normal commensal.
- Prominent cause of UTI in children and bacteracmia, which is attribuled to its urease production
- Motility and chemotaxis have role in pathogenesis
- Proteus differs from other enterobacteria by presence of enzyme phenylalanine deaminase (responsible for PPA reaction) which convert phenyl alanine to phenyl pyruvic acid.
- Culture of proteus bacilli have characteristic fishy or seminal smell.
- *P. mirabilis* and *P. vulgaris* swarms on solid culture media.
- **Antigenic structure** – Proteus posses flagelar (H) and somatic O antigen.
- **Infectious syndromes** – UTI (20-30% of complicated UTI); Pneumonia (primarily in hospitalized patient); Intraabdominal infections; soft tissue abscess.

Remember: Some strains of ***P. vulgaris*** called ***X strains*** are agglutinated by sera from typhus fever patient. This is due to sharing of carbohydrate hapten between ***Rickettsiae*** and proteus and forms basis of *Weil Felix reaction*.

Enterobacter

- Resemble klebsiella but are readily distinguished by their motility.
  - Ent. cloacae is the most important species.
  - Important cause of bacteraemia

Cronobacter

- C. sakazakii is an emerging pathogen associated with powedered milk causing necrotizing enterocolitis, sepsis, and meningitis in infants.
- Ampicillin and gentamicin is the treatment.

Multiple Choice Questions

E. Coli

1. **With reference to infection with Escherichia coli the following are true except:** [AI 05]
   a. Enteroaggregative *E. coli* is associated with persistent diarrhea
   b. Enterohemorrhagic *E. coli* cause hemolytic uremic syndrome
   c. Entero-invasive *E. coli* produces a disease similar to salmonellosis
   d. Entero toxigenic *E. coli* is a common cause of traveller's diarrhea

2. **A 20-year-old man presented with hemorrhagic colitis. The stool sample grow Escherichia coli in pure culture. The following serotype of *E. coli* is likely to be the causative agent:** [AI 04]
   a. 0 157:H7 b. 0 159:H7
   c. 0 107:H7 d. 0 55:H7

3. **A microbiologist wants to develop a vaccine for prevention of attachment of diarrheagenic *E. coli* to the specific receptors in the gastrointestinal tract. All of the following fimbrial adhesions would be appropriate vaccine candidates except:** [AI 04]
   a. CFA-1 b. Pi-Pili
   c. CS-2 d. K88

4. **All of the following are true about HUS except:**
   a. Infection may be transmitted by food [AI 2012]
   b. HUS is caused by serotoxin-producing *Escherichia coli*
   c. HUS is more common in children
   d. HUS is rarely associated with haemorrhagic colitis

5. **All are true about EHEC except:** [AI 01]
   a. Sereny test is positive
   b. Ferments sorbitol
   c. Causes HUS
   d. Elaborates Shiga like exotoxin

6. **All of the following are true except:** [AI 01]
   a. *E. coli* is an aerobe and facultative anerobe
   b. *Proteus* forms uric acid stones
   c. *E. coli* is motile by peritrichate flagella
   d. *Proteus* caused deamination of phenylalanine to phenylpyrivic acid

7. ***E. coli* attached to a surface with the help of:** [AI 00]
   a. Fucose b. Concanavalin
   c. Phytohemagglutinin d. Lactin

8. **Most common organism causing UTI:** [PGI 10]
   a. *E. coli* b. *Streptococci*
   c. *Klebsiella*
   d. Staphylococci saprophyticus

9. **A 20-year-old male had pain in abdomen and mild fever followed by gastroenteritis. The stool examination showed presence of pus cells and RBC's on microscopy. The most likely etiological agent is:**
   a. Enteroinvasive *E. coli* [AIIMS 03]
   b. Enteropathogenic *E. coli*
   c. Enterotoxigenic *E. coli*
   d. Enteroaggregative *E. coli*

10. **Which of these are true about *E. coli*:** [PGI 02]
    a. The L.T. (labile toxin), in ETEC acts via CAMP
    b. UTI causing *E. coli* attaches through pilli
    c. The ST (Stable toxin) of ETEC is responsible for causing hemolytic-uremic syndrome
    d. EIEC invasiveness is under plasmid control
    e. In EPEC, the toxin helps in invasion

11. **M.C cause of liver abscess:** [PGI 00]
    a. *Streptococcus* b. *Staph'aureus*
    c. *E. coli* d. *Staph pyogenes*

12A. **True about Enterotoxigenic E. Coli:** [AIIMS Nov 10]
    a. Causes epidemic diarrhoea in children in deve-loping countries
    b. Not a cause of traveller's diarrhoea
    c. Invasive
    d. Spread by contaminated water

12B. **Microbes with Et, Eh and EP strains:** [AIIMS Nov 14]
    a. E. coli b. Mycobacterium
    c. Shigella d. Neisseria

Salmonella

13. **In patient with typhoid, diagnosis after 15 days of onset of fever is best done by:** [AI 02]
    a. Blood culture b. Widal
    c. Stool culture d. Urine culture

14. ***Salmonella* typhi is the causative agent of typhoid fever. The infective dose of *S. typhi* is:**
    a. One bacillus [AIIMS 06, AI 2012]
    b. 10^8 - 10^{10} bacilli
    c. 10^2 - 10^5 bacilli
    d. 1 - 10 bacilli

15. **There has been an outbreak of food borne *Salmonella* gastroenteritis in the community and the stool sample is received in the laboratory. Which is the enrichment medium of choice:** [AIIMS 03]
    a. Cary-Blair medium
    b. V - R medium
    c. Selenite F medium
    d. Thioglycholate medium

16. **A 24 year cook in a hostel is suffering from enteric fever 2 years back. The chronic carrier state in patient is diagnosed by:** [AIIMS 02]
    a. Vi agglutination test
    b. Blood culture in brain heart infusion broth
    c. Widal test
    d. C. reactive protein

17. **For typhoid endemic country like India, immunization of choice is:** [AIIMS 01]
    a. TAB vaccine
    b. Typhoral 21A oral vaccine
    c. Monovalent vaccine
    d. Any of these

18. **Kallu, a 22-year-old male had an outing with his friends and developed fever of 38.5°C, diarrhea, and vomiting following eating chicken salad, 24 hours back. Two of his friends developed the same symptoms. The diagnosis is:** [AIIMS 01]
    a. *Salmonela enteritis* poisoning
    b. *Bacillus cereus*
    c. S. aureus
    d. *Vibrio cholera*

19. **True about salmonella gastroentritis is/are:** [PGI 06]
    a. Mainly diagnosed by serological tests
    b. Blood and mucous are present in stool
    c. Caused by animal products
    d. Symptoms appear between 4 - 48 hours
    e. Features are mainly due to exotoxin

20. **True about typhoid:** [PGI 03]
    a. It is caused by *S. typhi*
    b. Water can transmit the disease
    c. Type 21 a is an oral vaccine
    d. Chronic carrier is called when transmitted up to 6 month
    e. Widal test +ve in 1st week

21. ***Salmonella* gastroenteritis is:** [PGI 02]
    a. Mainly diagnosed by serology
    b. Blood and mucous present in blood
    c. Caused by animal products
    d. Symptoms appear by 4-48 hours
    e. Features are due to exotoxin released

22. **True about Salmonella typhi infection in intestine are:** [PGI 01]
    a. Affects peyer's patches
    b. Common in mesenteric border
    c. Erythrophagocytosis is characteristic
    d. Strictures are common
    e. Typhoid ulcer always bleed very much

23. **True about maximum isolation period of enteric fever:** [AIIMS 08]
    a. Till three consecutive negative urine/stool culture samples are obtained from patient.
    b. After chloramphenicol treatment for 72 hours.
    c. Disappearance of fever
    d. Widal test negative

24. **About Vi polysaccharide vacine, true is:**
    a. Can be given in patients with yellow fever and hepatitis B [AIIMS Nov 10]
    b. Has many contraindications
    c. Has many serious systemic side effects
    d. Has many serious local side effects

25. **True about salmonellosis:** [PGI 11]
    a. ↓ed incidence in developed countries
    b. Antacid and prolonged antibiotic administration promote infection
    c. Always fatal
    d. Food born to man and animal

Shigella

26. ***Shigella* can be differentiated from *E. coli by* all of the following features except:** [AI 99]
    a. *Shigella* does not produce gas from glucose
    b. *Shigella* does not ferment lactose
    c. *Shigella* does not ferment mannitol
    d. *Shigella* has no flagella and is non motile

27. **All are true about Shigella except:** [AI 2002]
    a. Large dose is required for infection
    b. Associated with hemolytic uremic syndrome
    c. Causes bloody diarrhea
    d. Gut pathology is due to toxin

Others

28. **HUS is caused by:** [PGI 07]
    a. EIEC
    b. *Shigella*
    c. *Salmonella*
    d. *Cholera*
    e. *Klebsiella*

29. **Which of the following is a true statement regarding Enterobacteriaceae:** [PGI 06]
    a. Motility is by polar flagellum
    b. Glucose is not fermented by all members of the family
    c. All members are oxidase positive
    d. Nitrate reduction negative

30. **Enterobacteriaecae includes all except:** [PGI 06]
    a. Pseudomonas
    b. *Klebsiella*
    c. V. cholera
    d. Proteus
    e. *E. coli*

31. **Maximum urease is produced by:** [PGI 00]
    a. *H. pylori*
    b. *P. Mirabilis*
    c. *K. rhinomatis*
    d. *Ureaplasma*

32. **DT 104 strain is belongs to which of the following bacteria:** [PGI Nov 11]
    a. *Salmonella Gallinarum*
    b. *Salmonella Typhi*
    c. *Salmonella Enteritidis*
    d. *Salmonella Paratyphi A*

Explanations and References with Illustrative Answers

1. **Ans. (c) Enteroinvasive *E. coli* produce a disease similar to Salmonellosis** *Ref. Ananthanarayan 8/e, 277, 9/e, p 279*

"Enteroinvasive E. coli produce disease similar to Shigellosis not salmonellosis."

Enteroinvasive E. coli (EIEC):

- Also called ***atypical E. coli*** as many strains don't ferment lactose or ferment it late.
- Resemble Alkalescens-Dispar Group
- Cause illness *similar to Shigellosis.*
- Ability to penetrate cells is due to presence of *plasmid* which *codes for virulence marker antigen (VMA).*
- **Diagnosis:** – Sereny test
  – Cell penetration of Hela or HEP. 2 in culture is diagnostic.

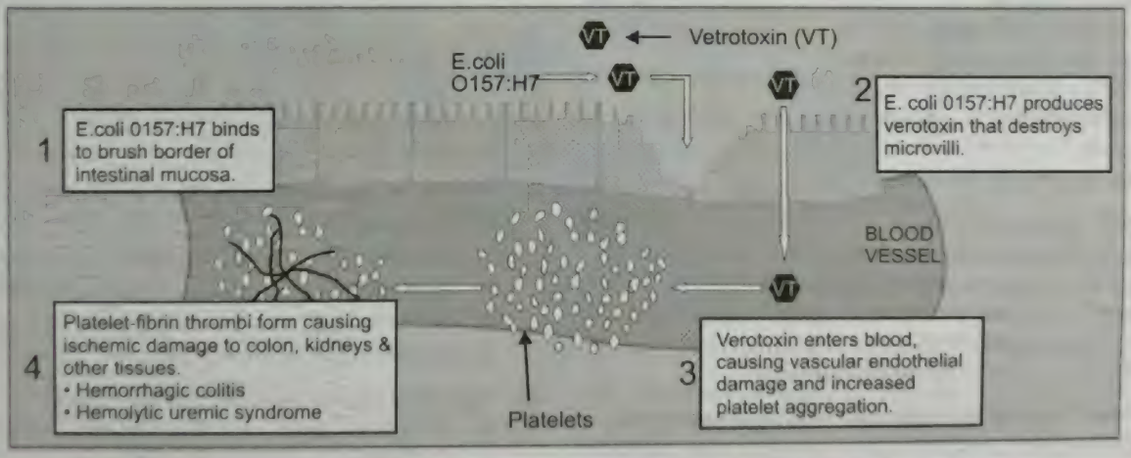
Remember:

• *Enteropathogenic E. coli* (EPEC)	– ***Diarrhea in infants and children***
• *Enterotoxigenic E. coli* (ETEC)	– ***Traveller's diarrhea***
• *Enteroinvasive E. coli* (EIEC)	– Diarrhea to dysentery ***similar to Shigellosis***
• *Enterohemorrhagic E. coli* (EHEC)	– ***Hemolytic uremic syndrome***
• *Enteroaggregative E. coli*	– ***Persistent diarrhea***

2. **Ans. (a) 0 157:H7** *Ref. Ananthanarayan 8/e, 277, 9/e, p 279; Harrison 19/e, p 1030, 18/e, p 1251*

"Typically O 157:H7 and few other such as O 26:H1 E.coli are associated with hemorrhagic colitis."

Pathogenesis of O157:H7



Enterohemorrhagic *E. coli* (EHEC) = Shigatoxigenic *E. coli* (STEC) = Verotoxigenic *E. coli* (VTEC):

- These strains produce verocytotoxin (VT) = Shiga like toxin (SLT).
- Cause diarrheal disease ranging in severity from mild diarhea to ***fatal hemmorhagic colitis*** and ***hemolytic uremic syndrome.***
- Primary ***target of VT is vascular endothelial cells.***
- VT is composed of an active A subunit and five B units that mediate binding
- *'A' subunit of Shiga toxin irreversibly inhibit ribosomal function.* ... *Harrison 17/e, p 940, 18/e, p 1251*
- Stx-2 mediated activation of complement may also play a role in the development of HUS.
- Typical EHEC is serotype 0 157:H7 and few others such as 0 26:H1.
- Diagnosis of EHEC diarrhea can be made by demonstration of bacilli or VT in feces.
- *Usually O 157:H7* serotype does *not ferment sorbitol* So, use of sorbitol Mac-Conkey medium helps in screening of O:157 VTEC.

3. **Ans. (b) Pi Pilli** *Ref. Jawertz 25/e, p 216; Harrison 17/e, p 937, 18/e, p 1246*

Correctly speaking guys we are unable to find all choices **but Pi Pilli is related in pathogenesis of pyelonephritis not GIT pathology.**

So, most probably that would be the answer.

4. **Ans. (d) HUS is rarely associated with haemorrhagic colitis** *Ref. Harrison 18/e, p 1251; 19/e, p 1030*

50% of all cases of HUS and 90% of HUS cases in children are caused by STEC/EHEC.

This complication is probably mediated by the systemic translocation of shiga toxin.

Furthermore HUS is only a rare uncommon complication of hemorrhagic colitis or else it can be said that most cases of HUS are associated with EHEC, but only few cases with hemorrhagic colitis progress to HUS.

It occurs in 2-8% of cases of hemorrhagic colitis.

Pathogenesis of HUS

- There is systemic translocation of Shiga toxin, where erythrocytes serve as carrier of stx to endothelial cells located in small vessels of kidney and brain.
- Subsequently there is thrombolic microangiopathy which manifest as combination of fever, thrombocytopenia, renal failure and encephalopathy.

5. **Ans. (a) Sereny test is positive** *Ref. Ananthanarayan 8/e, 277, 9/e, p 279*

"Sereny test is positive in cases of EIEC not EHEC."

Mostly 0 157:H7 serotype of EHEC don't ferment sorbitol but some 0157:H7 and non 0157 strain ferment sorbitol *so, option B (Ferments sorbitol) is partly correct.* ..... *Harrison 17/e, p 942*

Sereny test – Installation of suspension of freshly isolated *EIEC* or *Shigella* in to the eyes of guinea pigs leads to mucopurulent conjuctivitis.

6. **Ans. (b) *Porteus* forms uric acids stone** *Ref. Harrison 19/e, p 1033, 18/e, p 1254*

"Proteus results in formation of struvite stone not uric acid stone."

- Uric acid stone forms in acidic urine in presence of hyperuricemia.
- All members of Enterobacteriaceae are aerobes or facultative anaerobes.
- **Features of proteus bacilli:**
  - Non lactose fermenter
  - *Differs from other enterobacteria by presence of enzyme* phenylalanine deaminase which is responsible for deamination of phenylalanine to phenyl pyruvic acid (PPA test).
  - By producing urease it degrades urea to NH_3 and CO_2, NH_3 raises urinary pH. NH_4^+ (formed from NH_3) precipitate PO_4^{3-} Mg^{2+} to form $MgNH_4PO_4$ (Struvite.).
  - ***The result is stone of struvite admixed with $CaCO_3$***
  - This precipitation of organic compounds also contribute to the formation of biofilin on catheter. As proteus resides in biofilm and stones it can be eradicated only by removal of stones and/or catheter.
  - Some strains *(X-strains)* are agglutinated by sera from *typhus fever patient* (weil felix reaction).

Remember:
- Peritrichate flagella (flagella all around the cell) seen in:
  - *Proteus*
  - *E. coli*
  - *Listeria*
  - Clostridia
  - Typhoid bacilli
  - Bacillus
- PPA test is also seen in *Morganella, Providencia.*
- Urine samples with unexplained alkalinity should be cultured for proteus

7. **Ans. (a) Fucose** *Ref. Still searching*

I am unable to find the answer from any book. I am just mentioning the answer which is taken as correct by my seniors.

Remember: Concanavalin A and phytohemagglutinin is mitogen that cause blast transformation of T cells not of B cell.

8. **Ans. (a) *E. coli*** *Ref. Harrison 18/e, p 2388; 19/e, p 1028*

"E. coli cause 80% of acute UTI in patients with out catheterization." .....*Harrison 19/e, p 1028, 18/e, p 2388*

Strains of *E. coli* causing UTI are called extraintestinal pathogenic strains of *E. coli*.

Remember:
- *E. coli* is ***MC*** cause of catheter associate UTI too. ....*Harrison 17/e, p 1823, 18/e, p 2389*
- UTI is ***MC*** nosocomial infection.
- *E. coli* is ***MC*** cause of neonatal meningitis.
- *E. coli* is ***MC*** cause of intraabdominal abscess.
- **Other gram negative rods causing UTI are:** Proteus, klebsiella, serratia, pseudomonas, enterobacter.

9. **Ans. (a) Enteroinvasive (*E. coli*)** *Ref. Ananthanarayan 8/e 277, 9/e, p 278-279; Harrison 19/e, p 1031, 18/e, p 1252*

EIEC cause illness resembling Shigellosis i.e. ranging from mild diarrhea to frank dysentery. However Shigella, EIEC produce disease only with a large inoculum.

Causes of bloody diarrhea	
Organism	**Incubation period**
• Shigella	>16h
• EHEC	> 16h
• EIEC	>16h
• Campylobacter jejuni	2 - 6 days

10. **Ans. (a, b) and (d) The L.T., (labile toxin), in ETEC acts via cAMP; In types causing UTI the organism attaches through pilli; and EIEC invasiveness is under plasmid control** *Ref. Ananthanarayan 8/e, p 272 - 276, 9/e, p 276 - 279*

Enterotoxins of E. coli		
Stable Heat toxin	**Heat labile toxin**	**Verocytotoxin = Shiga like toxin**
• Acts through activation of CGMP	• Acts through activation of CAMP	• Inhibits protein synthesis

***Mnemonic* – Labile Toxin cAMP**

- Fimbriae (P fimbriae) or pilli binds to epthelium of urinary tract and helps in causing UTI.
- HUS is caused by verocytotoxin = Shiga like toxin of EHEC not by ST of ETEC.
- Invasiveness of EIEC is due to plasmid coated outer surface antigen called virulence marker antigen.
- EPEC or Enteroadherent E.coli causes diarrhea by disruption of brush border not by toxin or invasion.

11. **Ans. (c) *E. coli*** *Ref. Harrison 17/e, p 938, 18/e, p 1249; 19/e 1028-1030*

Remember: ***E.coli* is *MC* cause of:** – Intraabdominal abscess – Neonatal meningitis – UTI.

Note: Most common manifestation of *E. coli : Diarrhea > UTI > Abdominal and pelvic infection*

12A. **Ans. is (d) Spread by contaminated water** *Ref. Ananthanarayan 8/e 276, 9/e, p 279*

Enterotoxigenic *E. coli* (e.g. 06, 08, 015, 025, 027, 0167)

- Affect *all age group. ETEC diarrhea is endemic in the developing countries*
- *MC* cause of traveller's diarrhea (indistinguishable from cholera).
- First step of pathogenesis is adherance of bacteria to intestinal mucosa which is mediated by colonisation factor antigen
- After adhesion bacteria produce either or both LT and STa. However, toxin alone does not lead to diarrhea.
- *Source of infection:* Contaminated water or food.

12B. **Ans. is (a) E. coli** *Ref. Ananthanarayan 9/e 276*

Already explained

13. **Ans. (b) Widal test** *Ref. Ananthanarayan 8/e, 294 - 296, 9/e, p 298*

" In 3rd week Widal test is investigation of choice."

Diagnosis of Typhoid

I. **Cases (BASU)**

Blood culture (B):	– Test of choice in first week (Diagnostic gold standard). – Becomes negative on treatment with antibiotics.
Widal test:	– Agglutinins usually appear by end of first week. Titre increase steadily till the 3rd or 4th week after which it declines gradually. – Maximum titre is found in 3rd week.
Stool culture (S)	– *Salmonella* are shed in feces throughout the course of disease, even in convalescence. A positive fecal culture occur in carriers also, so can't differentiate between case and carrier.
Urine culture:	– Culture is positive in 25% of cases during 2nd or 3rd week. – Less useful than blood culture.

II. **Carriers**

- *Widal* reaction – ***No value*** in detection of carriers.
- Demonstration of ***Vi agglutinins indicate carrier state.*** This is a usefull screening test which is confirmed by culture.
- Isolation of bacillus from feces or bile. Cholagogue purgatives increase chance of isolation.
- Tracing of carriers in cities is done by 'Sewer Swab Technique' or by filtration of sewage through millipore membrane and *culturing the membrane* on *Wilson* and *Blair media.*

Widal reaction:

- Test for measurment of H and O agglutinins for typhoid and paratyphoid bacilli in patient serum.
- Agglutinins usually appear by the end of 1st week. Titre ↑ steadily till the 3rd or 4th week. Two types of tubes are generally used for the test –a narrow tube with the conical bottom (Dreyer's agglutination tube) for H agglutination and a short round bottomed tube (Felix tube) for O agglutination.
- **O Agglutinins** – O antigen is common between S. typhii and S. paratyphii, so doesn't specify whether infection is due to S. typhii or S. paratyphii.
  - It has better diagnostic value than H.
  - O antibodies disappear after infection.
  - O agglutination is seen as disk like pattern at bottom of tube.
  - Titer > 1 : 320 is significant.
- **H agglutinins** – H antigen are different for *S. typhii* or *S. paratyphii*, so indicates type of infection
  - May be present due to prior disease, inapparent infection, thus it does not have good diagnostic value.
  - Persist longer than O agglutinins.
  - Titer > 1: 640 is considered positive
  - Reaction to it, is rapid and leads to formation of cotton wooly clumps.
  - More immunogenic

Remember: Order of disappearance of antibody in typhoid $V_1 \rightarrow O \rightarrow H$.

Widal test is false positive in case of prior infection and immunization.

14. Ans. (c) 10^2 - 10^5 bacilli *Ref. Harrison 19/e, p 1049, 18/e, p 1274*

Infective dose of salmonella varies from 200 to 10^6 colony forming units and the ingested dose is an important determinant of incubation period

Organism	Infective dose
Shigella	10 - 1000
Vibrio	$>10^{10}$ (if source of infection is water) 10^2 - 10^4 (if source of infection is food)
Campylobacter jejuni	10^4
Yersinia enterocolitica	10^8 - 10^9
EHEC	10 - 100

15. Ans. (c) Selenite 'F' medium *Ref. Ananthanarayan 8/e 289, 299, 9/e, p 291, 300*

"Selenite F and Tetrathionate broth are commonly employed enrichment media of salmonella."

***Salmonella* Gastroenteritis = Food poisoning**

- May be caused by **any Salmonella except *S. typhi***
- ***MC*** caused is ***S. typhimurium***
- Human infection occurs due to ingestion of contaminated foods.
- **Most frequent source** – Poultry, meat, milk and milk products
- **IP** – 16 - 48 hours
- ***Clinical feature*** : Diarrhea, vomiting, fever
- ***Diagnosis*** : Isolation of salmonella from feces
- ***Treatment*** : No antibiotics

Remember:

Differential media for *Salmonella*:

- *MacConkey and Deoxycholate media:* Form *colorless* colonies due to absence of lactose fermentation.
- *Wilson and Blair bismuth sulphite medium: Jet black* colonies are formed due to production of H_2S.
- *Selective media for salmonella:* SS agar, Deoxycholate citrate agar.

16. Ans. (a) Vi. agglutination test *Ref. Ananthanarayan 8/e 290, 9/e, p 292-293*

Vi antigen – *Polysaccharide antigen enveloping the O antigen because of which many strains of S. typhi fails to agglutinate with the 'O' antiserum.*

Antigens of Salmonella		
H antigen	O antigen	Vi. antigen
– Present on flagella	– Integral part of cell wall	– Envelops the 'O' antigen
– Heat labile protein	– Phospholipid - protein - polysaccharide complex	– Surface polysaccharide
– Strongly immunogenic	– Identical with endotoxin so, is less immunogenic	– Acts as virulence factor and is poorly immunogenic
– Antibody formation is rapid and in high titre	– Antibody formation is slow and titre is low	– Antibody production is slow and titres is low

Normally Vi antibody disappears early in convalescence. Its persistance indicates the development of carrier state.

Note: Total absence of Vi antibody in a proven case of typhoid indicates poor prognosis.

Chronic carriers of *S. typhi:*

- Persons who excrete bacilli for more than a year after clinical attack.
- Average carrier rate is 3% (i.e. 3% cases become chronic carriers).
- Carrier state is more common in females (cases are more common in males).
- Fecal carriers are more frequent than urinary carriers but urinary carriers are more dangerous.
- Urinary carrier state is often associated with some abnormality of the urinary tract.
- Fecal carrier stage is *more common* in patients with biliary abnormality and GI malignancy.
- ***Diagnosis of carriers:*** – Demonstration of Vi antigen
  – Isolation of *S. typhi* by sewer swab technique.
- ***Treatment:*** – Ampicilin (4-6 g a day) together with probenecid for 6 week.
  – Cholecystectomy with concomitant ampilcillin therapy has been regarded as the MOST sucessfull approach to treatment of carriers. ... *Park 22/e, p 215*

17. Ans. (c) Monovalent vaccine *Ref. Park 22/e, p 215*

Since *S. typhi* is the major cause of typhoid fever in India, the vaccine of choice is the monovalent typhoid vaccine.

Antityphoid vaccine are:

a. ***Monovalent antityphoid vaccine*** – Heat killed and phenol preserved.

b. ***Bivalent antityphoid vaccine*** – Contains *S. typhi* and paratyphi A.

c. ***TAB vaccine (WHO recommended that TAB vaccine should be discontinued).***

d. ***Live oral typhoid 21a vaccine (Typhoral)***
   - Enteric coated capsule of lyophilized vaccine containing not less than 10 viable organism of attenuated *S. typhi* strain Ty 21a. S. typhi strain Ty21A lacks the enzyme UDP-galactose - 4 epimerase (GALE Mutant)
   - It is indicated for immunization of adults and children aged more than 6 years.
   - ***Protection commences 2 weeks after taking last capsule and last for at least 3 years***
   - **Dose** - 1 capsule on days 1, 3 and 5 one hour before meal with cold or luke warm milk or water.
   - Ingestion, Ty21a strain initiates infection but self destructive after four or five cell divisions and therofpre can not induce my illress.

e. **Typhin V_1 Vaccine (V_1CPS)**
   - Injectable vaccine contains purified Vi polysaccharide antigen (25 µg/dose) from S.typhi strain Ty^2. Vaccine is injected as single subcutaneous or IM injection, which causes only minimal local reaction (Mild pain, erythma).
   - Provides immunity for 2 years.
   - It is contraindicated in patients with a history of hypersensitivity to any components.
   - Because of T-cell independent properties, it is poorly immunogenic in children < 5 years of age.

f. **VirEPA Vaccine**
   - Recently developed (not yet marketed) vaccine in which Vi is bound to a nontoxic recombinant protein that is identical to Pseudomonas aeruginosa exotoxin A. It is more immunogenic than ViCPS vaccine.

18. Ans. (a) *Salmonella* enteritis poisoning *Ref. Ananthanarayan 9/e, p 301; Harrison 17/e, p 960, 18/e, p 1279*

• IP of *S. aureus* food poisoning	– 1-6 hours	
• IP of *B. cereus* food poisoning	– Diarrhea	– 8 to 16 hours.
	– Vomiting	– 1 to 6 hours.
• IP of *V.cholera* food poisoning	– > 16 hours.	

19. **Ans. (c) and (d) Caused by animal products and symptoms appear between 4 - 48 hours** *Ref. Ananthanarayan 8/e, p 299, 9/e, p 300*
Already explained

20. **Ans. (a, b) and (c) It is caused by *S. typhi* , Water can transmit the disease and Type 21 a is an oral vaccine**
Ref. Ananthanarayan 8/e, p 293 - 297, 9/e, p 295 - 300
- Typhoid is caused by *S.typhi*.
- Water is mode of transmission.
- Ty 21a is a live oral vaccine.
- Chronic carriers are those who excrete bacilli for more than a year (not 6 months).
- Widal test gives negative results in 1st week.

21. **Ans. (c) and (d) Caused by animal products and symptoms appear by 4 - 48 hrs** *Ref. Ananthanarayan 8/e, 299, 9/e, p 300*
Already explained

22. **Ans. (a) Affects Peyer's patches** *Ref. Harrison 19/e, p 1049, 18/e, p 1276*
Enteric manifestations of *S.typhi*:
- *S. typhi* ***invades peyer's*** patches and form ***oval ulcer*** with their long axis along the length of bowel (Tuberculosis result in transverse ulcer).
- Peyer's patches lie along antimesentric border, so ulcer are common in antimesentric border.
- Stricture is rare but ***perforation can occur (Stricture are common in TB ulcer).***

Remember: Erythrophagocytosis is feature of *E. histolytica*.

Neurologic manifestations of enteric fever:
- Meningitis
- GB syndrome
- Neuropsychiatric symptoms (described as muttering delirium or coma vigil)

23. **Ans. (a) Till three...** *Ref. Park 22/e, p 214*

Control of Typhoid Fever
- Control of reservoir:
  Reservoir may be case or carrier:
  A. **Cases:**
  - *Early diagnosis and treatment:* With early diagnosis and treatment, carrier stage can be prevented.
  - *Isolation:* Owing to the infectious nature of typhoid fever, cases should be isolated till three bacteriologically negative stools and urine reports are obtained on three separate days.
  - *Disinfection:* Stools and urine are sole source of infection. They should be received in closed containers and disinfected with ***5% cresol*** for at least 2 hours. All solid clothes and linen should be soaked in a solution of 2% chlorine and steam sterilized. All the medical and paramedical person should disinfect their hands.
  - *Follow-up:* Follow up examination of stool and urine should be done 3-4 months after discharge of patient and again after 12 months to prevent the development of carrier stage.

  B. **Carriers**
  - *Identification:* Culture of duodenal drainage establishes the presence of *Salmonella* in the biliary tract in carriers. The Vi antibody is seen in up to 80% of carriers.
  - *Treatment:* Ampicillin or amoxycillin (4-6 g a day) together with probenecid can achieve eradication of carrier stage in about 70%.
  - *Cholecystectomy with concomitant ampicillin therapy* is the ***most successful*** approach for treatment of carriers.
  - *Surveillance:* The carriers should be kept under surveillance and are prevented from handling food, milk for others.
- **Control of Sanitation:**
  - The weakest link in the chain of transmission is sanitation which can be achieved easily
  - Protection and purification of drinking water supplies, improvement of basic sanitation and promotion of food hygiene are essential measures of controlling typhoid.
- **Immunization:**
  - Immunization does not give 100% protection, but it definitely lowers both the incidence and seriousness of infection.

24. **Ans. is (a) can be given in .....** *Ref. Harrison 19/e, p 1052; 18/e, 1278*
Yellow fever is not a contraindication for VICPS typhoid vaccine.

25. **Ans. (a) (b) and (d) ↓ ed incidence in developed countries, Antacid and prolonged antibiotic administration promote infection, Food born to man and animal** *Ref. Ananthanarayan 8/e, 288-96, 9/e, 295-300; Harrison 18/e, p 1274-1276*
Let's consider each option.

Option 'a'

- With improvement in food handling and water sewage treatment enteric fever has become rare in developed nations.
- **Incidence is :**
  - Highest (> 100 cases per 100,000 population) in north central and southeast Asia
  - *Medium* (10-100 cases per 100,000 population) in the rest of Asia, Africa, Latin America and Oceania (including Australia and New Zealand).
  - Low (≥ 10 cases per 100,000 population) in the rest of world.

Option 'b' ...*Harrison 18/e, p 1274; 19/e, p1050*

Conditions that increase susceptibility to salmonella infection

a. *Reduced gastric acidity :* Age < 1 year, antacid ingestion, achlorhydria
b. *Loss of intestinal integrity:* Inflammatory bowel disease, prior gastrointestinal surgery
c. *Alteration in intestinal flora* by prior antibiotic administration

Option 'c' Case fatality rate of enteric fever is < 1%

Option 'd' Salmonellosis is a food borne or water borne disease resulting from ingestion of food or water contaminated by feces of case or carrier.

Option 'e' Bacilli are killed at 55°C in one hour or at 60°C in 15 minutes. Culture may be viable for years if prevented from drying. *Ref. Ananthanarayan 8/e, 289, 9/e, 291*

26. Ans. (c) Shigella does not ferments mannitol *Ref. Ananthanarayan 8/e, 283, 9/e, p 286*

Differentiating features between *Shigella and E.coli*		
Features	**Shigella**	***E. coli***
Motility	—	+
Lactose fermentation	—	+
Glucose fermentation	+ (Produce acid)	+ (Produce acid and gas)
Lysine decarboxylase	—	+

- ***Shigella ferment mannitol and this property is used for its classification.***

Classification:

Mannitol fermenters	Non fermenters
S. flexneril	*S. dysenteriae*
S. boydii	
S. sonnei	

So, guys mannitol fermentation can't diferentiate E. coli and Shigella as E.coli also ferments mannitol.

Remember:
- *S. sonnei* **is late lactose fermenter.**
- All members of Enterobacteriaceae are motile except *Shigella, Klebsiella* and *Salmonella* gallinarum-pallorum.
- *E. coli* also ferments maltose.

27. Ans. (a) Large dose is required for infection *Ref. Ananthanarayan 7/e, p 287, 9/e, p 287; Harrison 17/e, p 962*

Correctly speaking 2 choices are wrong i.e. ***option "a" and option "d".***

- ***Infective dose for bacillary dysentery is just 10 to 100 bacilli as Shigella survive gastric acidity better than other enterobacteria.***

So, *option "a"* is clearly wrong.

- ***Sh. dysenteraie forms enterotoxin*** *(acts by inhibiting protein synthesis)* ***which appears to be less important in pathogenesis than invasive property.*** *However Shiga toxins are translocated from bowel into the circulation. After binding to the cells in kidney, toxin may lead to homolytic uremic syndrome.*

Thus, ***option "d"*** is also wrong but not completely.

So, Answer would be clearly wrong ***option*** **i.e.** ***"a"***

Remember: Small infective dose (10 - 100 bacilli) required in EHEC, *Entamoeba, Giardia.*

28. Ans. (b) ***Shigella*** *Ref. Harrison 19/e, p 1056; 18/e p 1283*

Two important complication of shigellosis are HUS and toxic megacolon.

29. Ans. (b) Glucose is not fermented by all members of the family *Ref. Ananthanarayan 8/e 272, 7/e, p 274*

"Members of enterobacteriaceae reduce nitrates to nitrites, form catalase but not oxidase."

Features	Enterobacteriaceae: Important features		
	Escherichia	*Salmonella*	*Shigella*
Motility by peritnchous flagella	+	+	–
Gas from glucose	+	+	–
Acid from lactose	+	–	–
Acid from sucrose	d	–	–
Growth in KCN	–	d	–
Indole	+	–	d
MR/VP	+	+	+
Citrate	–	+	–
H2S	–	+	–
Urease	–	–	–
Phenylalanine deaminase (PPA)	–	–	–
Arginine dehydrolase	d	+	–
Lysine decarboxylase	+	+	–
Ornithine decarboxylase	d	+	d

(d = result different in different species or strains)

Remember: – *Polar flagella*: Vibrio, pseudomonas, legionella, spirilla, campylobacter, *H. pylori*, spirochetes.
– V. parahemolytic produce both polar and peritrichous flagella.

30. **Ans. (a and c) *Pseudomonas*; and *V. cholerae*** *Ref. Ananthanarayan 8/e 271, 9/e, p 273-274*

Enterobacteriaceae			
Tribe I	***Tribe II***	***Tribe III***	***Tribe IV***
Escherichiae	***Klebsiellae***	***Protecae***	***Erwinieae***
Genus • *Escherichia* • *Edwardsiella* • *Citrobacter* • *Salmonella* • *Shigella*	Genus • *Klebsiella* • *Enterobacter* • *Hafnia* • *Serratia*	Genus • *Proteus* • *Morganella* • *Providencia*	Genus • *Erwinia*

31. **Ans. (b) *P. mirabilis*** *Ref. Ananthanarayan 8/e, p 279, 9/e, p 282*

Urease production (in order of decreasing):
– ***Proteus*** – ***Klebsiella*** – ***Citrobacter***

32. **Ans. (e) *Salmonella Typhimurium*** *Ref. Harrison 18/e, p 1278*

DT-104 is a multidrug resistant strain of S. typhimurium which emerged in early 1990. It is associated with increased risk of blood stream infection and hospitalization. Humans acquired infection through exposure to ill farm animals land to various meat products.

Chapter Review

1. **Enteropathogenic *E. coli*:** **[AIIMS 90]**
   a. Causes acute gastroenteritis in infants
   b. Invades intestinal epithelium
   c. Produces cholera like toxin
   d. It is non-motile
   [Ref. Ananthanarayan 8/e, p 276, 9/e, p 279]

2. **Following are true of Widal test except:** **[AI 92]**
   a. High titre in first sample is diagnostic
   b. 'H' antigen is specific for serotyping
   c. Highly + ve H titre not diagnostic
   d. Maximum titre in third week
   [Ref. Ananthanarayan 8/e, p 296, 9/e, p 298]

   High titre in first sample can be due to prior infection or immunization. Rising titre in two or three samples is more specific.

3. **The following are gas producing *Salmonella* except:**
   a. *S. typhi* b. *S. enteritidis* **[AI 92]**
   c. *S. cholera* d. *S. typhimurium*
   [Ref. Ananthanarayan 8/e, p 289, 9/e, p 296]

   All *Salmonella* except *S. typhi* ferment glucose, mannitol and maltose, forming acid and gas; S. typhi is anaerogenic.

4. **Agglutination with 'O' antigen of *S. typhi* is inhibited by:** **[AIIMS 92]**
   a. Vi antigen b. Pili antigen
   c. Flagellar antigen d. All of the above
   [Ref. Ananthanarayan 8/e, p 290, 9/e, p 293]

5. **Typhoid carriers are detected by following except:** **[UP 06]**
   a. Isolation of bacteria from urine
   b. Isolation of the bacteria from bile
   c. "Vi" antigen
   d. Widal test *[Ref. Park 19/e, p 197]*

6. **Prolonged *Salmonella* septicemia is caused by:** **[PGI 93, 94]**
   a. *S. enteritidis* b. *S. cholerae-suis*
   c. *S. typhimurium* d. *S. typhi*
   [Ref. Ananthanarayan 8/e, p 300, 9/e, p 301]

   S. choleraesus cause septicemic disease with focal suppurative lesions such as osteomyelitis, deep abscesses, endocarditis, pneumonia and meningitis. The case fatality may be as high as 25%.

7. **Which is true of Enterobacteriacae:** **[AI 93]**
   a. All are oxidase negative
   b. Nitrate reduction negative
   c. Glucose not fermented by all
   d. Motility bipolar flagellum
   [Ref. Ananthanarayan 8/e, p 270, 9/e, p 273]

8. **"Pea-soup stool" is characteristically seen in:**
   a. Cholera b. Typhoid **[DNB 04]**
   c. Botulism diarrhea d. Traveller's diarrhea
   e. Salmonellosis
   [Ref. Ananthanarayan 8/e, p 332]

9. **All of the following are true regarding typhoid except:**
   a. Urinary carriers are more dangerous **Kerala 04]**
   b. Vi Ab is used for detecting carrier
   c. Vi is seen is normal population
   d. Urine carrier is associated with anomalies
   [Ref. Ananthanarayan 8/e, p 290, 9/e, p 297]

10. ***In vitro* test for enterotoxin** **[Manipal 04]**
    a. Mc Conckey's culture
    b. Blood agar culture
    c. Rabbit ileal loop culture
    d. None
    [Ref. Ananthanarayan 8/e, p 274, 9/e, p 277]

    ***In vitro* test for ETEC include:**
    - Tissue culture tests
    - Rounding of Y_1 mouse adrenal cells
    - Elongation of CHO cells
    - ELISA
    - Passive agglutination test
    - Genetic tests (DNA probes)

11. **All are true about enterohemorrhagic *E. coli* except:**
    a. Sereny test positive **[AIIMS 99]**
    b. May cause diarrhea
    c. Can cause hemolytic uremic syndome
    d. Verocytotoxin is produced
    [Ref. Ananthanarayan 8/e, p 277, 9/e, p 279]

12. **Which of the following is false about Widal?** **[Delhi 08]**
    a. O agglutinins has better diagnosic value than H
    b. O antibodies disappear after infection
    c. First titre is confirmatory
    d. H agglutinins persist longer than O agglutinins
    [Ref. Ananthanarayan 8/e, p 296, 9/e, p 298]

Answers 1. a. Causes ... 2. a. High titre ... 3. a. *S. typhi* 4. a. Vi antigen 5. d. Widal test
6. b. *S. cholerae* ... 7. a. All are ... 8. b. Typhoid 9. c. Vi is seen is ... 10. d. None
11. a. Sereny ... 12. c. First titre ...

13. Which subtype of *E. Coli* causes hemorrhagic colitis?
a. 0157:H7 b. 026:H1 **[Delhi 08]**
c. Both of the above d. None of the above
[Ref. Ananthanarayan 8/e, p 277, 9/e, p 279]

14. Incubation period of typhoid is: **[Delhi 08]**
a. 10-14 days b. 2-6 days
c. 18-72 hours d. 2-3 months
[Ref. Ananthanarayan 8/e, p 293, 9/e, p 295]

15. Widal test is a: **[Delhi 08]**
a. Slide agglutination test
b. Flocculation test
c. Tube agglutination test
d. Complement fixation test
[Ref. Ananthanarayan 8/e, p 108, 9/e, p 298]

16. HUS is associated with: **[AP 06]**
a. EAEC b. EIEC
c. EHEC d. EPEC
[Ref. Ananthanarayan 8/e, p 277, 9/e, p 279]

17. Labile toxin of *E. coli* can be detected by the following methods of incubation except: **[Kerala 2K]**
a. Into infant rabbit bowel
b. Into adult rabbit' skin
c. Intra gastrically into infant mouse
d. Into tissue culture of chinese hamster ovary cells
e. Into YI mouse adrenal cells
[Ref. Ananthanarayan 8/e, p 274, 7/e, p 276]

18. True about widal test is: **[UP 00]**
a. Widal test confirmative in endemic area
b. Antibiotic treatment does not alter widal test
c. Previous infection affects Widal test
d. Does not alter with prior vaccination
[Ref. Ananthanarayan 8/e, p 296, 9/e, p 298]

19. All of the following are true regarding typhoid except:
a. Urinary carriers are more dangerous **[AIIMS 99]**
b. Vi ab is used for detecting carrier
c. Vi is seen in normal population
d. Urine carrier is associated with anomalies
[Ref. Ananthanarayan 8/e, p 290, 9/e, p 293, 296]

20. True about typhoid is: **[UP 00]**
a. Incubation period 3-6 weeks
b. Chronic carrier is 10-15%
c. Widal test is specific
d. Vi polysaccharide of bacterial cell used for vaccination
[Ref. Ananthanarayan 8/e, p 299, 9/e, p 300]

21. Correct statement about Widal test is: **[Kolkata 02]**
a. Only O antigen is used
b. Is a tube agglutination
c. Any antibody titre is diagnostic
d. Antibody appears after 1-10 days of fever
[Ref. Ananthanarayan 8/e, p 296, 9/e, p 109]

22. Which toxin is mediated by C-AMP except:
a. *V. cholera* 01 **[AIIMS 98]**
b. Heat stable *E. coli* toxin
c. Heat labile *E. coli* toxin
d. *V. cholera* 0137
[Ref. Ananthanarayan 8/e, p 273, 9/e, p 277]

23. Microorganisms that enter freshly laid eggs are:
a. *Salmonella* b. *Brucella* [Kar 03]
c. *Shigella* d. *Vibrio cholerae*
[Ref. Ananthanarayan 8/e, p 299]

24. All of the following *Salmonella* are motile except:
a. *S. typhi* b. *S. enteridis* **[SGPGI 04]**
c. *S. gallinarum* d. *S. chester*
All salmonella are motile by peritrichate flagella except for S. gallinarum and S. pullorum.
[Ref. Ananthanarayan 8/e, p 289, 9/e, p 291]

25. Widal test is an example of: **[DNB 05]**
a. Flocculation b. Agglutination
c. Both d. None
[Ref. Ananthanarayan 8/e, p 296, 9/e, p 109]

26. Non-lactose fermenter includes all the following except: **[MP 07]**
a. *Shigella sonnei* b. *Shigella dysenteriae*
c. *Shigella flexneri* d. *Shigella boydii*
[Ref. Ananthanarayan 8/e, p 270, 9/e, p 286]

27. Persistant diarrhea is caused by: **[UP 07]**
a. EAEC b. EIEC
c. ETEC d. EPEC
[Ref. Ananthanarayan 8/e, p 277, 9/e, p 279]

28. Verocytotoxin of *E. coli* acts by: **[BHU 07]**
a. Increasing cAMP
b. Decreasing cAMP
c. Decreasing protein synthesis
d. Decreasing cGMP
[Ref. Harrison 18/e, p 1251]

29. True statement about Widal test in typhoid is:
a. O-antigen titre remians positive for several months and reaction to it is rapid [AI 99]
b. H-antigen titre remains positive for several months and reaction to it is rapid
c. Both remains positive for several months and reaction to both is rapid
d. None
[Ref. Ananthanarayan 8/e, p 298, 9/e, p 298]

Answers 13. c. Both of the ... 14. a. 10-14 days 15. c. Tube ... 16. c. EHEC 17. c. Intra ...
18. c. Previous ... 19. c. Vi is seen... 20. d. Vi polysaccha... 21. b. Is a tube... 22. b. Heat stable ...
23. a. Salmonella 24. c. S. gallinarum ... 25. b. Agglutination 26. a. *Shigella* 27. a. EAEC
28. c. Decreasing ... 29. b. H-antigen...

30. True statement about widal test in typhoid is:
a. Widal test is confirmative in endemic areas
b. Antibiotic treatment does not alter widal test results
c. Previous infection alters widal test **[AI 99]**
d. Widal test does not alter with prior vaccination
[Ref. Ananthanarayan 8/e, p 296, 9/e, p 298]

31. Which of the following statement regarding *Shigella dysenteriae* type I is true: [AI 99]
a. It can lead to hemolytic uremic syndrome
b. It produces an invasive enterotoxin
c. It is an facultative aerobes
d. It is MR negative
[Ref. Ananthanarayan 8/e, p 284, 9/e, p 287]

32. Traveller's diarrhoea, is caused by: [AIIMS 97]
a. *Shigella*
b. *E. coli*
c. *E. histolytica*
d. Giardiasis *[Ref. Ananthanarayan 8/e, p 276, 9/e, p 279]*

Cause of Traveller's diarrhea		
Bacterial	***Viral***	***Parasitic***
ETEC (**MC**) *V. cholera* *Shigella* *Salmonella* *C. jejuni*	Rotavirus (**MC**) Norwalk virus	*Giardia* (**MC**) *Entameba histolytica* *Cryptosporodium* *Cyclospora*

33. Which of the following is true about Enteropathogenic *E. coli*: [AI 96]
a. Causes diarrhea in infants
b. Acts by invasion of intestinal epithelial cells
c. Adults are mostly affected
d. Affects immunocompromised host
[Ref. Ananthanarayan 8/e, p 276, 9/e, p 279]

34. Which of the following is true regarding *Salmonella* infection: [AI 96]
a. Urine culture is +ve in 1st week
b. Stool culture is +ve in 1st week
c. Blood culture is +ve in 3-7 days
d. Widal test is +ve in 1st week
[Ref. Ananthanarayan 8/e, p 294-296]

35. Which of the following produces enterotoxin: [AI 95]
a. *Sh. dysenteriae*
b. *Sh. sonnei*
c. *Sh. flexneri*
d. *Sh. boydii*
[Ref. Ananthanarayan 8/e, p 285, 9/e, p 286]

Exotoxins associated with diarrheal diseases are called as enterotoxins. They are produced by:

– *Shigella dysenteriae I*	– *Staph. aureus*
– *B. cereus*	– *Cl. perfringes*
– *Y. enterocolitica*	– *V. parahemolyticus*
– V. cholera	– **ETEC**
– *Kleibsella pneumonia*	– *Aeromonas*

36. Which antigen blocks the agglutination of *salmonella* by 0 antiserum: [AI 95]
a. H.
b. *Fimbriae*
c. Vi
d. 0
[Ref. Ananthanarayan 8/e, p 289, 9/e, p 293]

37. A person returns to Delhi from Bangladesh after 2 days and has diarrhea. Stool examination shows RBCs in stool. The likely organism causing is:
a. Enteropathogenic *E. coli* **[AIIMS 99]**
b. Enterotoxigenic *E. coli*
c. Salmonella typhi
d. *Shigella dysenteriae*
[Ref. Ananthanarayan 9/e, p 287]

38. *E. coli* gives pink color with: [PGI 99]
a. Chocolate agar
b. L J medium
c. MacConkey's medium
d. Saline broth
[Ref. Ananthanarayan 8/e, p 270, 7/e, p 271]

Being Lactose fermenter E.coli give pink color with MacConkey's medium.

Answers **30.** c. Previous ... **31.** a. It can ... **32.** b. E. coli ... **33.** a. Causes ... **34.** c. Blood...
35. a. Sh. dysenteriae **36.** c. Vi **37.** d. Shigella... **38.** c. MacConkey's

NEET Pattern Questions

1. **Watery diarrhea in children is caused by:**
   a. ETEC
   b. EPEC
   c. EIEC
   d. EAEC
   [Ref. Ananthanarayan, 9/e, p 279; Harrison 18/e, p 1249]

2. **MC cause of diarrhea in children of developing country is:**
   a. EHEC
   b. ETEC
   c. EACL
   d. EIEC

3. **Performed toxin is important in food poisoning due to all except:**
   a. Staph aureus
   b. Clostridium botulism
   c. ETEC
   d. B. cereus *[Ref. Ananthanarayan, 9/e, p 279]*

ETEC first adheres with E. coli and then secretes exotoxin which causes diarrhea.

4. **In E. coli true is:**
   a. ETEC is invasive
   b. EPEC acts via cAMP
   c. Pilli present in uropathogenic type
   d. ETEC causes HUS *[Ref. Ananthanarayan, 9/e, p 277]*

Strains carrying K antigens are more commonly responsible for pyelonephritis while most isolates from cystitis lacks K antigen. The P pili-positive strains are uropathogenic E. coli.

5. **A child with fever with RBCs and pus in stools, causative organism is:**
   a. ETEC
   b. EHEC
   c. EPEC
   d. EAEC *[Ref. Ananthanarayan, 9/e, p 279]*

6. **In donovanosis:**
   a. Pseudolymphadenopathy
   b. Penicillin is used for treatment
   c. Painful ulcer
   d. Suppurative lymphadenopathy

Donovanosis (or Granuloma inguinale)
- Veneral disease caused by Klebsiella granulomatis
- IP: 1-12 weeks
- Disease begins as a painless papule on genitalia which progress to auto inoculable ulcer and runs a chronic course
- Diagnosis is confirmed by demonstration of donovan bodies in Wright-Giemsa stained smears
- They may show bipolar condensation of chromatin, giving a closed safety pin appearance
- Treatment of choice is tetracycline

7. **Recommended transport medium for stool specimen suspected to contain enteric pathogens is:**
   a. Arnie's medium
   b. Buffered glycerol saline medium
   c. MacConkey medium
   d. Blood agar *[Ref. Ananthanarayan, 9/e, p 297]*

8. **Phenylalanine deaminase test is positive in:**
   a. Salmonella
   b. Proteus
   c. Vibrio cholerac
   d. Helicobacter *[Ref. Jawetz, 27/e, p 233]*

9. **Enteric fever is caused by:**
   a. S typhi
   b. S paratyphi A
   c. S paratyphi C
   d. All of the above *[Ref. Harrison, 19/e, p 1050]*

10. **Selective medium for shigella:**
    a. Chocolate agar
    b. BYCE medium
    c. Hekton agar
    d. EMJH medium
    [Ref. Harrison, 19/e, p 1057]

11. **Indicator used in MaConkey Agar:**
    a. Methylene blue
    b. Methyl red
    c. Neutral red
    d. Bromothymol blue

Mac Conkey's agar is a selective, indicator and differential medium. It contains peptone, agar, sodium taurocholate and neutral red. Lactose fermenters form pink colonies while non-latose fermenters produce colourless or pale calories.

Answers 1. b. EPEC 2. b. ETEC 3. c. ETEC 4. c. Pilli present in uropathogenic type
5. b. EHEC 6. a. Pseudolymphadenopathy 7. c. MacConkey medium
8. b. Proteus 9. d. All of the above 10. c. Hekton agar 11. c. Neutral red

12. Shiga toxin acts by:
a. Activating adenylyl cyclase to increase cAMP
b. activating guanylyl cyclase to increase cGMP
c. Inhibiting protein synthesis
d. Inhibiting DNA replication
[Ref. Ananthanarayan, 9/e, p 287]

13. Shigella are be divided into subgroup on the basic of ability to ferment:
a. Lactose b. Maltose
c. Fructose d. Mannitol
[Ref. Ananthanarayan, 9/e, p 286]

Fermenation of mannitol is of importance in classification and shigella have traditionally been divided in to mannital fermenting and non fermenting species. S.dysenteriae being non-fermenting one.

14. True about Shiga toxin:
a. An endotoxin
b. Inhibit protein synthesis
c. Activate adenylyl cyclase
d. Increase cGMP *[Ref. Ananthanarayan, 9/e, p 287]*

Exotoxin of shigella is also known as shiga token or verotoxin note. LPS of wall of shigella acts as endotoxin.

15. True about widal test:
a. Anti-O antibody persists longer
b. O antigen of S. paratyphi is used
c. H-antigen is most immunogenic
d. Felix tube is used for 'H' agglutination
[Ref. Ananthanarayan, 9/e, p 292, 298]

Antigens used for widal test are the H and O antigens of S. typhi and the H. antigens of S. Paratyphi A and D
(for details see answer no 13 in explanatory answers)

16. Salmonellae other than S. typhi and S. paratyphi cause:
a. Typhoid fever
b. Enteric fever
c. Gastroenteritis
d. All of the above *[Ref. Ananthanarayan, 9/e, p 297]*

Clinical manifestations of non-typhoidal salmonellosis
- Gastroenteritis
- Bacteremia and endovascular infection
- Localized infection (Intra-abdominal abscess, meningitis, pulmonary injection, UTI)
- Bone joint and soft tissue infection

17. Chronic carrier of typhoid shed bacilli for:
a. 1-3 weeks after cure
b. 3 weeks to 3 months after cure
c. 3 months -1 year after cure

Convalescent carrier: Excrete bacilli for 6-8 weeks.
Chronic carrier. Person who excrete bacilli for more than a year.

18. Culture media used for O157 : H7 enterohemorrhagic E. coli:
a. Sorbitol containing agar
b. Mannitol containing agar
c. Sucrose containing agar
d. Dextrose containing agar
[Ref. Ananthanarayan, 9/e, p 278]

19. Enterobacteriaceae is classified based on:
a. Mannitol fermentation
b. Catalase and oxidase reaction
c. Oxygen requirement
d. Lactose fermentation *[Ref. Ananthanarayan, 9/e, p 271]*

20. Shiga toxin is preduced by:
a. Enteropathogenic E. coli
b. Enteroinvasive E. coli
c. Enterohemorrhagic E. coli
d. Enterotoxigenic E. coli
[Ref. Ananthanarayan, 9/e, p 279]

21. Salmonella infection is most commonly caused by:
a. Infected water
b. Infected vegetable
c. Aerosol infection
d. Through skin

22. Infective dose of Salmonella typhi:
a. 10 bacilli b. 1000 bacilli
c. 10^3–10^6 bacilli d. 10^{10}–10^{12} bacilli
[Ref. Ananthanarayan, 9/e, p 285]

23. Not true about Vi polysaccharide vaccine of typhoid:
a. Single dose is given
b. Revaccination at 3 years
c. Given at birth
d. Given subcutaneously *[Ref. Ananthanarayan, 9/e, p 300]*

24. Salmonella and shigella can be differentiated from other enterobacteriaceae member by isolation on:
a. MacConkey agar
b. Mannitol salt agar
c. BCYE medium
d. XLD agar *[Ref. Ananthanarayan, 9/e, p 285]*

Answers **12.** c. Inhibiting ... **13.** d. Mannitol **14.** b. Inhibit protein ... **15.** c. H-antigen... **16.** c. Gastroenteritis
17. d. More than ... **18.** a. Sorbitol cont... **19.** d. Lactose ... **20.** c. Enterohemorrhagic E. coli
21. a. Infected water **22.** c. 10^3–10^6 bacilli **23.** c. Given ... **24.** d. XLD agar

25. Clinical significance of Vi antigen of S. typhi is:
a. Helps in diagnosis
b. Highly immunogenic
c. Most important antigen for widal test
d. Antibody against Vi-antigen is used for diagnosis of carrier *[Ref. Ananthanarayan, 9/e, p 299]*

26. All are important causes of UTI except:
a. E. coli
b. Proteus
c. Klebsiella
d. Streptococcus viridans *[Ref. Harison, 19/e, p 862]*

27. Proteus isolated from a patient of UTI will show which boichemical reaction:
a. Phenylpyruvic acid reaction
b. Bile esculin reaction
c. Colchicine sensitivity
d. Bacitracin sensitivity *[Ref. Ananthanarayan, 9/e, p 282]*

28. Most common cause of hemolytic uremic syndrome is:
a. E. coli
b. Shigella
c. Salmonella
d. Psedononas *[Ref. Harison, 19/e, p 1862]*

29. Hebra nose is caused by:
a. Frisch bacillus
b. Staph aureus
c. Pseudomonas
d. C. diphtheriae

30. Most common cause of pyelonephritis in pregnancy:
a. Pseudomonas
b. E. coli
c. Proteus
d. Klebsiella

31. Absence of Vi-antibody in a typhoid patient has:
a. Good prognosis
b. Bad prognosis
c. No relation with prognosis
d. Indicates widal negative

32. Which of the following is late lactose fermenter:
a. E. coli
b. Klebsiella
c. Salmonella
d. Shigella sonnei

Answers 25. d. Antibody... 26. d. Streptococcus... 27. a. Phenylpyruvic... 28. a. E. coli 29. a. Frisch bacillus
30. b. E. coli 31. b. Bad prognosis 32. d. Shigella sonnei

CHAPTER

16

Vibrio

- Gram-negative, rigid, motile curved rods. **All are halophilic except *V. cholerae* and *V. mimicus*.**
- They are ***oxidase positive***, which differentiates it from gram-negative enteric bacteria.
- They are susceptible to compound ***0/129*** which differentiate them from *Aeromonas species*.

..... *Jawetz 25/e, p 236*

I

Vibrio

Motile, Gr (–) ve rods that are oxidase positive and give a positive indole reaction.

- Prefer alkalinity, require salt.
- TCBS is the best selective media whereas alkaline peptone water is the best enrichment medium.

Vibrio Cholera

- Comma shaped, isolated by Koch.
- Arranged as ***fish in stream appearance.***
- Posses single polar flagella and shows *darting type motility (= swarm of gnats).*
- Natural habitate is coastal salt water and brackish estuaries where organism lives in close association with plankton.

Culture Characteristics

- Grows well on ordinary media.
- Strongly aerobic, better growth in alkaline medium (pH 8.5 - 9.5) and are rapidly killed by acid.
- ***Required NaCl*** *(0.5-1%)* for optimal growth; however, *6% and above are inhibitory.*
- ***On MacConkey's agar:*** Late lactose fermenters.
- ***On Gelatin Stab:*** Infundibuliform (funnel shaped) or napiform (turnip shaped) liquification occurs.

	Special media	
Holding or transport media	**Enrichment media**	**Plating media**
• VR medium • Cary-Blair medium also for *Shigella* and *Salmonella* • Autoclaved sea water	• Alkaline peptone water at a pH of 8.6 • Monsour's taurocholate tellurite peptone water • Both used as transport media when specimen reach labortories within few hours.	• Alkaline bile salt agar • Monsour's gelatin taurocholate trypticose tellurite agar (GTTA) • TCBS: *Best selective media*

- Colonies are identified by *string test.*
- ***Vibrios are suceptible to heat drying but resist high alkalinity***

Biochemical characterstics

C : Catalase +ve
O : Oxidase +ve
I : Indole +ve
N : Nitrates reduced to nitrites
S : Sucrose fermenter

] Responsible for ***cholera red*** reaction

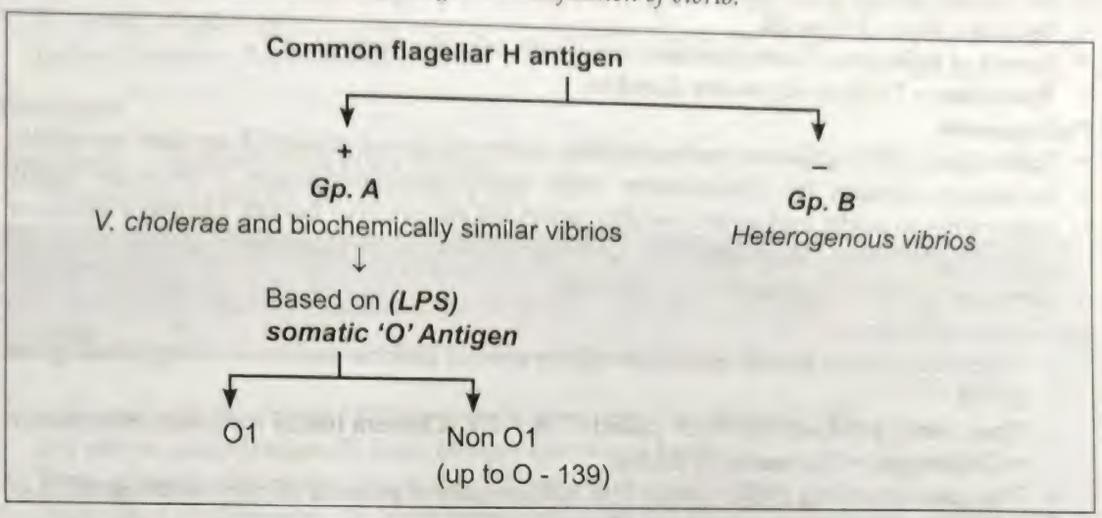
Enzymes:
- Neuraminidase [receptor destroying enzyme]
- Lipase
- Chitinase
- Elastase
- Mucinase

Classification

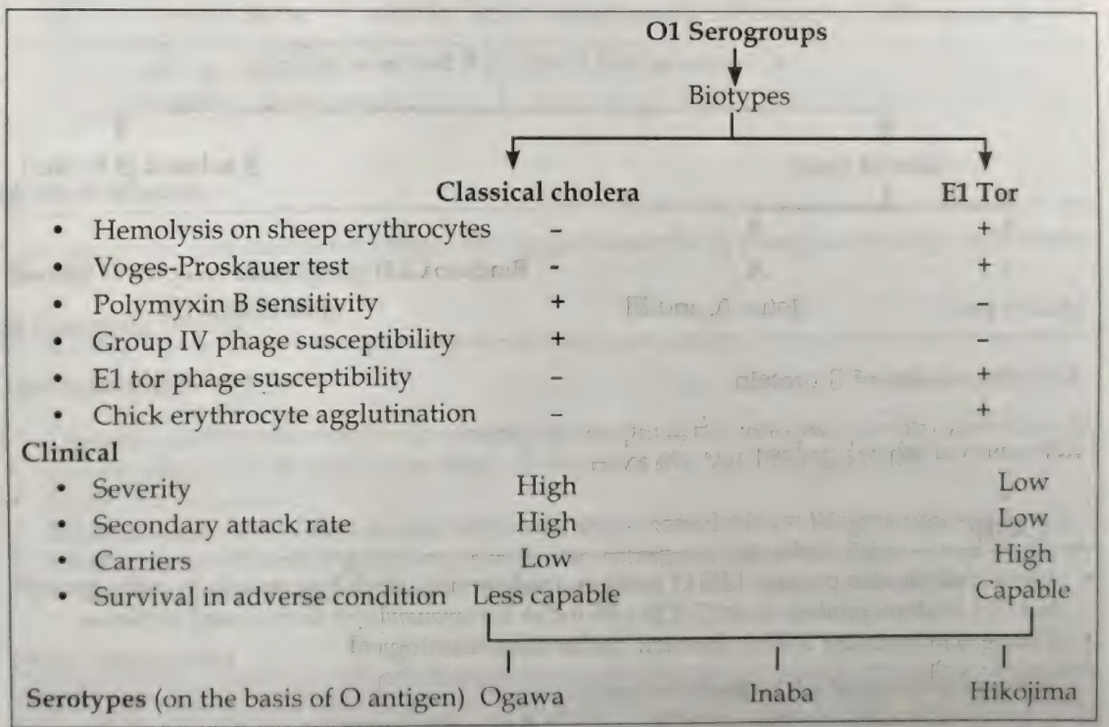
I

Classified on the basis of lipopolysaccharide O-antigens into V.cholera OI and Non-OI V.cholerae.
- Flagellar antigen is same in all V. cholerae
- Ogawa serotype of EI tor is the MC strain of vibrio, causing cholera

- *Gardner and Venkatraman's Serological Classification of vibrio:*



- Only O1 serogroup cause cholera till 1992, so Non O1 serogroup were known as Noncholera Vibrio (NCV) or Non-Agglutinable Vibrios (NAG vibrios).
- The latest serogroup O -139 identified in 1992 causes epidemic of cholera emphasising that they can not longer be considered as noncholera vibrios.



- ***Ogawa serotype of E 1 tor is MC*** strain causing cholera [7th Pandemic].
- Ogawa and Inaba strain are agglutinated by their own antisera while hikojima is agglutinated by both Ogawa and Inaba antisera.
- ***O-139 strain is called Bengal V. cholera*** (arise from E1 tor by *horizontal gene transfer*) signaled begininig of 8th pandemic. It differs from E1 tor in production of Of-139 LPS and an immunologically related O antigen polysaccharide *capsule.* ..... *Harrison 19/e, p 1062*

Remember: *V. cholerae* belong to group I of ***Heiberg grouping of vibrios***.

- V. cholerae O-139 is associated with free living aquatic amoebae and other members of zooplankton which acts as reservoir of infection.

Cholera

- ***Incubation period*** – 1-2 days.
- ***Infective dose*** – 10^6 bacilli.
- ***Source of infection*** – Water contaminated with infective feces.
- ***Symptoms*** – Painless rice water diarrhea.

Pathogenesis

- Individuals with *O group are most susceptible* white with blood group *AB- are least susceptible.*
- In human infection, the vibrios enter orally via contaminated water. Vibrios are highly susceptible to acids, and gastric acidity provides an effective barrier against vibrios, or else achlorhydria is a significant risk factor for cholera, so in salmonella typhi.
- Cause *non invasive toxin* mediated diarrhea.

Mechanism of Diarrhea

- Vibrio adheres to jejunal epithelial cell by special fimbria and toxin coregulated pilus [TCP].
- Then vibrio produces exotoxin called CTX = **CT (Cholera toxin)** = cholera enterotoxin = Choleragen = Permeability factor.
- The genes encoding cholera toxin (ctx AB) are part of genome of bacteriophage CTX φ.
- CT can be demonstrated by 'Skin blueing test'.
- CT *inhibits absorption of Na^+ and Cl^-* as well as, ↑ secretion of K+ and HCO_3; resulting in isotonic diarrhea, acidosis with elevated anion gap (*due to increase in serum lactate protein*). *..... Harrison 19/e, p 1062*
- CT also ↑ intestinal secretion via prostaglandins and neural histamine receptors.
- It has no effect on any other tissue except intestinal cells.

CT (similar to LT of *E. coli* but more potent)

- A subunit (one)
  - A_1 (Active part) → ADP ribosylation of G protein → Activation of adenyl cyclase → ↑ CAMP
  - A_2 (Joins A_1 and B)
- B subunit (5 in No.) → Binds to GM1 ganglioside receptor of **jejunal epithelial cells.**

- *Vibrio cholerae* also possess LPS O Antigen ***(endotoxin)*** which has ***no role*** in pathogenesis, but *O-139 strain produce novel O-139 LPS which is responsible for its increased virulence.*
- Changes in intestine are biochemical rather than histological.

Virulence Factor of 01 V. cholerae are:

- CTx including gene encoding CTx which are part of genome of bacteriophage CTXϕ.
- TCP
- Vibrio cholerae may express one or more of four haemolysin: Thermostable direct hemolysin, E1 Tor haemolysin, thermolabile haemolysin and thermostable hemolysin. The last three are found in pathogenic strains where they contribute to virulence.

Lab diagnosis

- **Specimen:**
  - Rectal swabs for convalescent phase.
  - ***Stool collected*** by introducing a lubricating catheter into rectum is best specimen.
  - Rapid diagnosis by characteristic **darting motility** and its inhibition by antiserum under the dark field or phase contrast microscope.

I

V. Cholerae exhibit two major pathogenic mechanism:
1. Produce cholera toxin.
2. Express toxin co-regulated pilli.

Cholera toxin acts by activating adenylate cyclase.

I

Period of communicability
Case: 7-10 days
Convalescent carries: 2-3 weeks
Chronic carriers: A month to more than 10 years

- *Serological examination:*
  - Helpful in assessing prevalence of cholera in an area. Complement dependent vibriocidal antibody test is most useful. Other include antitoxin assay, indirect hemagglutination.
- For examination of water sample for vibrios, enrichment or filtration method used.
- In carrier stage antibody titer against V. cholerae raises and remain positive as long as person harbours the organisms.

..... *Park 22/e, p 209*

Treatment

Treatment of choice: Rehydration therapy

- For **mild to moderate** dehydration: **ORS**

Composition of WHO ORS						
ORS	Na^+	Cl^-	K^+	Citrate	Glucose	Osmolalrity
m mol/l	75	65	20	10	75	245

..... *Harrison 18/e, p 1293*

  - If available rice based ORS is considered superior to standard ORS.
- For **severe** dehydration: IV fluid ***(Ringer Lactate is best).***
- ***Drug of choice for adults****: Single dose tetracycline or doxycycline.*
  Alternative erythromycin. In areas where tetracycline resistance in prevelant, ciprofloxacin is recommended.
- **For children & pregnant mother :** Erythromycin or Azithromycin (10 mg/kg).
- **Control:** Water sanitation and proper excreta disposal are the most effective control measure.

Note: Bacteria with safety pin appearance
- Yersinia pestis
- Vibrio parahaemolyticus
- Burkholderia mallei
- Klebsiella granulomatis
- Haemophilus ducreyi

Vibrio Mimicus

Nonhalophilic, nonsucrose fermenter causing *gastroenteritis* by eating *seafood* especially oyster.

Halophilic Vibrios

I

Halophilic vibrios
- Vibrios, that require salt for growth
- Includes:
  - V. parahemolyticus
  - V. alginolyticus
  - V. vulnificus.

Vibrio parahaemolyticus

- Capsulated vibrio showing *bipolar staining* (safety pin appearance)with peritrichous flagella.
- Grows only in media containing NaCl. ***It tolerates 8% NaCl*** but not > 10%.
- String test is positive.
- ***Exhibits Kanagawa Phenomenon*** (ability to show hemolysis on Wagatsuma agar).
- Cause ***gastroenteritis (= food poisoning)*** after eating sea fish (shell fish).
  - Cause of enteritis is ***invasion*** not enterotoxin.

Vibrio alginolyticus

- ***Most salt tolerant*** species of cholera. Can tolerate > 10% NaCl.
- Cause infection of eye, ear and wounds exposed to sea water.

Vibrio vulnificus

VP negative lactose fermenter (but not sucrose) that has a salt tolerance of less than 8%

Cause: a. Primary sepsis in patient with underlying liver disease.
b. Primary wound infection without underlying disease.

Multiple Choice Questions

1. **All of the following are true about *V. cholerae* O139 except:** [AI 08]
   a. Clinical manifestations are similar to O1 E1 tor
   b. First discovered in Chennai
   c. Produces O139 lipopolysaccharide
   d. Epidemiologically indistinguishable from O1 E1 tor

2. **Which of the following bacteria acts by increasing cAMP:** [AI 07; AIIMS 06]
   a. *Vibrio cholerae*
   b. *Staphylococcus aureus*
   c. *E. coli* heat stable toxin
   d. *Salmonella*

3. **About *V. cholerae* all statements are true except:**
   a. Nonhalophilic [AI 07]
   b. Cannot grow in ordinary media
   c. Cansurvive outside the intestine
   d. Man is the only reservoir of cholera

4. **Not true about vibrio O139:** [AI 07]
   a. Can cause disease in distinguishable from E_{-1} or clinically
   b. First isolated in Chennai
   c. Has O polysaccharide capsule
   d. Antibody to *V. cholerae* is not protective against O139

5. **All of the following *Vibrio species* are halophilic except:** [AI 05]
   a. *V. cholerae* b. *V. parahemolyticus*
   c. *V. alginolyticus* d. *V. flovialis*

6. **In the small intestine, cholera toxin acts by:** [AI 05]
   a. ADP ribosylation of G regulatory protein
   b. Inhibition of adenyl cyclase
   c. Activation of GTPase
   d. Active absorption of NaCl

7. **Antibiotic treatment of choice for treating cholera in an adult is a single dose of:** [AI 05]
   a. Tetracycline b. Cotrimoxazole
   c. Doxycycline d. Furazolidone

8. **The effect of cholera toxin is mediated via the stimulation of following second messenger:** [AI 2012]
   a. cAMP b. cGMP
   c. Calcium-calmodulin d. Acetylcholine

9. **Selective media for vibrio:** [AIIMS 08]
   a. TCBS b. Stuart
   c. Skirrows d. MYPA

10. **The best suited medium for *Vibrio cholerae* is:**
    a. Thayer martin [AIIMS 07]
    b. TCBS medium
    c. Scirrow medium
    d. Loeffler's medium

11. **The endotoxin of the following gram-negative bacteria does not play any part in the pathogenesis of the natural disease:** [AIIMS 06, AI 2012, AIIMS Nov 2012]
    a. *Escherichia coli*
    b. *Klebsiella sp.*
    c. *Vibrio cholerae*
    d. *Pseudomonas aeruginosa*

12. **Cholera toxin:** [AIIMS 06]
    a. Increases the levels of intracellular cyclic GMP
    b. Acts through the receptor for opiates
    c. Causes continued activation of adenylate cyclase
    d. Inhibits the enzyme phosphodiesterase

13. **The drug of choice for treating cholera in pregnant women is:** [AIIMS 05]
    a. Tetracycline b. Doxycycline
    c. Furazolidone d. Cotrimoxazole

14. **Which of the following is the drug of choice for chemoprophylaxis of cholera?** [AIIMS 05]
    a. Tetracycline b. Doxycycline
    c. Furazolidone d. Cotrimoxazole

15. **True about *V. cholerae* is:** [AIIMS 02]
    a. One attack of *V. cholerae* gives life-long immu-nity
    b. Affects adults and children with equal propensity in nonepidemic regions
    c. In between epidemics, carrier states maintain the organism
    d. Pathogenicity of 0-139 vibrio is due to O antigen

16. **A 32 years old male, Kallu who recently visited a sea coast presented with ulcer over the left leg. The probable cause is:** [AIIMS 01]
    a. *Pasteurella multocida* b. *Micrococcus halophillus*
    c. *Vibrio vulnificus* d. *Neisseria gonorrhea*

17. **All of the following statements about E1-Tor Vibrios are true, Except:** [AI 2010]
    a. Human are the only reservour
    b. Can survive in ice cold water for 2-4 weeks
    c. Killed by boiling for 30 seconds
    d. Enterotoxin can have direct effects on other tissues besides intestinal epithelial cells.

18. **Which toxin acts by ADP ribosylation:** [PGI 07]
    a. Botulinum toxin b. *Shigella toxin*
    c. *V. cholerae* d. Diphtheria toxin
    e. *Pertussis*

19. ***V. cholerae* able to stay in GIT because of:** [PGI 06]
    a. Acid resistance b. Bile resistance
    c. Motility d. Binds to specific receptors
    e. Anaerobic potential

20. Cholera transmission by: [PGI 06]
a. Food transmits
b. Vaccination gives 90% efficiency
c. Healthy carrier
d. Chlorination is not effective

21. In patient presenting with diarrhea due to vibrio cholera, which of the following will be present:
a. Abdominal pain [PGI 05, 01]
b. Presence of leukocytes in stool
c. Fever
d. Neutrophilia
e. Occurrence of many cases in the same locality

22. True about epidemiology of cholera: [PGI 03]
a. Chemoprophylaxis is not effective
b. Boiling of water cannot destroy the organism
c. Food can transmit the disease
d. Vaccination give 90% protection

23. Strain of *V. cholerae* in Bengal: [PGI 04]
a. 0:037 b. 0:139
c. 0:17 d. 0:40
e. 0:149

24. In a patient presenting with diarrhea and pus cells in stool, the causative organism can be all except:
a. *Non-vibrio cholerae* 01 [PGI 01]
b. Enterotoxigenic *E. coli*
c. Enteroinvasive *E. coli*
d. *Shigella dysenteriae* 1
e. *Vibrio cholerae*

25. Cholera is caused by? [PGI June 09]
a. *Vibrio cholerae* .01 b. *Vibrio cholerae* 0139
c. *V. parahemolyticus* d. *E. coli*
e. NAG *vibrio*

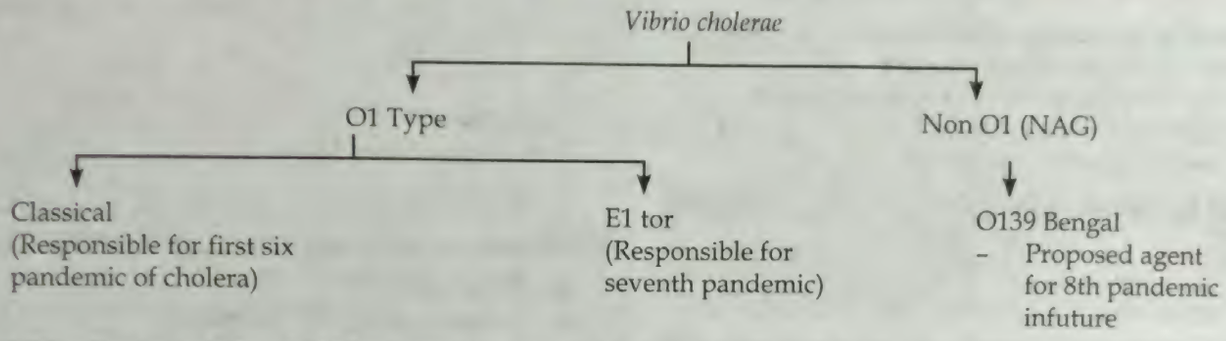
26. A stool examination was carried out which showed organism with darting motility. Which of the following organism may be in stool: [PGI May 2013]
a. V. cholerae
b. Shigella
c. Salmonella
d. Camplyobacter jejuni
e. E. coli

27. True about Vibrio alginolyticus: [PGI May 2013]
a. Non-halophilic
b. Voges Proskauer (VP) positive
c. Swarming
d. Cause sea borne auricular infections
e. Does not grow in 10% NaCl

Explanations and References with Illustrative Answers

1. **Ans. (b) First discoverd in Chennai** *Ref. Harrison 17/e, p 969, 18/e, p 1290; 19/e, p 1062*

Guy's before solving this question, you must understand the epidemiology of cholera.



In 1992, after its isolation O139 had initially replaced E_1 tor and become the most common causative agent of cholera in South Asia. However, by the beginning of 1994 E_1 tor had resumed its dominance in many areas and above all 0139 has not spreaded outside Asia.

- *O139 Vibrio was isolated in Bengal in 1992.*
- The clinical manifestation and epidemiological features of the disease caused by *V. cholerae* O139 are indistinguishable from those of O1 cholera.
- ***O139* V. cholerae *is identical to* E_1 *tor except for two important differences:***
  - Production of novel O139 Lipopolysaccharide.
  - Immunologically related O antigen polysaccharide capsule.

Remember: *E_1 tor biotype* was first isolated at the E_1 tor quarantine station in *Egypt* in 1905.
– ***Presently most cases of cholera are caused by E_1 tor.***

2. **Ans. (a) *Vibrio cholerae*** *Ref. Harrison 17/e, p 970, 18/e, p 1290; 19/e, p 1062*

Cholera toxin (CT)

- Protein *enterotoxin*
- Composed of 2 subunit: **A** – (Active monomeric moiety)
  B – (Pentameric binding moiety)
- **Mechanism of action:**

The B-pentamer binds to GM_1 ganglioside, a glycolipid on the surface of epithelial cells that serves as toxin receptor
↓
The activated A subunit (A_1) irreversibly transfers ADP ribose from NAD to regulatory component of adenyl cyclase
↓
Up regulation of adenylate cyclase
↓
Intracellular increase in cAMP

In intestine cAMP inhibits the absorptive sodium transport system in the villus cells and activates the secretory chloride system in the crypt cells. Thus, increased cAMP leads to leads to accumulation of sodium chloride in the intestinal lumen. To maintain osmolality water moves passively to the lumen and isotonic diarrhea starts.

Note: Though perturbation of the adenylate cylase pathway is the primary mechanism of cholera; cholera toxin also enhances the intestinal secretions via prostaglandins and/or neural histamine receptors.

Remember: *Mechanism of action* of some important bacterial toxin.

– Heat labile toxin of E. coli	– ↑ cAMP
– Heat stable toxin of E. coli	– Increase cGMP
– Botulism toxin	– Inhibit release of acetylcholine from peripheral nerves.
– Tetanus toxin	– Inhibit release of glycine and GABA at presynaptic terminals.
– Diphtheria toxin	– Inhibit protein synthesis by inactiviting EF-2.

3. **Ans. (b) Cannot grow in ordinary media** *Ref. Ananthanarayan 8/e, p 302; 9/e, p 304; Park 22/e, p 208*
   - Cholera grows well on ordinary media.
   - Growth is better on alkaline medium. NaCl is required for optimal growth though high concentrations are inhibitory.
   - *Option "c"* – Natural habital of *V. cholerae* is coastal salt water and brackish estuaries, where the organism live in close relation to plankton.
     - Human become infected incidentally, but once infected can act as vehicle for spread.
     - Man is the only reservoir of cholera infection. He may be the case or carrier.

Carriers in cholera			
Preclinical or convalescent carrier		***Contact or healthy carrier***	***Chronic carrier***
Incubatory	– Last for 2-3 weeks after attack	– Result from subclinical infection	– Can excreate up to 10 years.
		– Duration less than 10 days	– Gallbladder is infected
		– Gallbladder is not infected	

4. **Ans. (b) First isolated in Chennai** *Ref. Harrison 17/e, p 969, 18/e, p 1290; 19/e, p 1062*

   Already explained, refer Ans. 1

5. **Ans. (a) *V. cholerae*** *Ref. Ananthanarayan 8/e, p 312, 9/e, p 311*

   Halophilic vibrios:
   - Vibrios that have high requirement of NaCl.
   - All vibrios are halophilic except *V. cholerae* and *V. mimicus.*
   - Natural habitat of halophilic vibrios is sea water.

   Disease caused are:

V. parahemolyticus	Gastroenteritis; wound infection
V. vulnificus	Sepsis (in immunocompromised); secondary cellulitis
V. alginolyticus (Most halophilic)	Wound infections, cellulitis

6. **Ans. (a) ADP ribosylation of G regulatory protein** *Ref. Harrison 17/e, p 970, 18/e, p 1291*

   Already explained

7. **Ans. (c) Doxycycline** *Ref. Harrison 18/e, p 1293; 19/e, p 1063*

   "Doxycycline in single dose is the antibiotic of choice for adults (excepting pregnant women)."

   Antibiotic treatment of Cholera

   WHO recommends administration of antibiotic to cholera patients only if they are severely dehydrated.
   - ***Doxycycline*** is the *antibiotic of choice* for ***adults*** (excepting pregnant women).
   - Pregnant woman and children are usually treated with erythromycin or azithoromycin.
   - ***Ciprofloxacin*** is the recommended agent in area where doxycycline resistance is prevalent.

   Chemoprophylaxis – Tetracycline is *DOC* for chemoprophylaxis

8. **Ans. (a) cAMP** *Ref. Harrison 18/e, p 1291; 19/e, p 1062*

   Already explained

9. **Ans. (a) TCBS** *Ref. Ananthanarayan 8/e, p 310, 9/e, p 309*
   - *TCBS (media containing thiosulphate, citrate, bilesalts, sucrose)* is the best ***selective media*** for vibrio.
   - Vibrio produces yellow convex colonies.
   - In holding or transport media, Vibrios do not multiply but remain viable.

Holding or transport media	Plating media
1. VR medium	1. Alkaline bile salt agar
2. Caryblair medium	2. GTTA
3. Alkaline peptone water*	3. TCBS (best selective media)
4. Monsur's taurocholate tellurite water*	4. Mac Conkey agar (Non-selective)
***are also enrichment media**	

Mnemonic

Transport media – *Venkatraman carry alkaline peptone water to Maysoore* (Monsour).

Biochemical characteristics – *[COINS]* – Catalase +ve; – Oxidase +ve; – Indole +ve; – Nitrate reducer – Sucrose fermenter

10. **Ans. (b) TCBS medium** *Ref. Ananthanarayan 8/e, p 310, 9/e, p 309*

Already explained

11. **Ans. (c)** ***Vibrio cholerae*** *Ref. Ananthanarayan 8/e, p 306 - 307, 9/e, p 308*

Beside cholera toxin, *V. cholerae* also posses the lipopolysaccharide O antigen (LPS endotoxin) which apparently plays no role in pathogenesis of cholera but is responsible for the immunity induced by killed vaccine.

Other options:

E. coli – Pathogenesis is mediated by endotoxin, adhesins, capsule present in some strain, enterotoxin.

Pseudomonas – Exotoxin produce tissue necrosis by blocking protein synthesis.
– Endotoxin plays a role in causing fever, shock, oligouria, leukocytosis, DIC, ARDS.

Klebsiella – Pathogenesis is mediated by endotoxin and fimbriae or other adhesin.

Remember: – Endotoxin levels can be assayed by 'Limulus test'.
– Plague toxin also has no role in natural disease.

12. **Ans. (c) Causes continued activation of adenylate cyclase** *Ref. Harrison 17/e, p 970, 18/e, p 1290, 19/e, p 1062*

Already explained

13. **Ans. (c) Furazolidone** *Ref. Park 18/e, p 181; 19/e, p 193*

Antibiotics used in the **treatment of cholera.**

Condition	Antibiotic
Adult except pregnancy	Doxycycline once
Chemoprophylaxis	Tetracycline 4 times a day for 3 days
Children & Pregnancy	Erythramycin or Azithromycin

In older days furazolidone was used to treat pregnant women. As this question is old one, so the answer was furazolidone.

14. **Ans. (a) Tetracycline** *Ref. Park 22/e, p 211*

Already explained

15. **Ans. (d) Pathogenicity of 0-139 vibrio is due to O antigen** *Ref. Harrision 18/e, p 1270; 19/e, p 1063*

- Vibriocholerae 0:139 Bengal is identical to E_1 tor ***except for:***
  - Production of the novel ***0-139 LPS***
  - Presence of immunologically related O antigen polysaccharide ***capsule.***
- Both of these ***acts as virulence factor*** and explain resistance of 0-139 strain.
- In between epidemics the organism is maintained in their natural habitat, i.e. water.

16. **Ans. (c)** ***Vibrio vulnificus*** *Ref. Ananthanarayan 8/e, p 313, 9/e, p 312*

V. vulnificus:

- Halophillic vibrio
- Natural habitat is sea water
- Cause two **types of illness:**
  - In ***normal host :*** Wound infection following contact of open wound with sea water.
  - In ***immunocompromised host*** *(particularly with liver disease)* : Sepsis.

17. Ans. (d) Enterotoxin can have *Ref. Park 20/e, p 208*

Cholera enterotoxin does not exert extraintestinal effects.

Resistance of bacteria to surroundings

- Highly susceptible to heat, drying and acids. Destroyed at 55°C in 15 minutes, boiling kill, vibrio in few seconds.
- They can remain in ice for 4-6 for weeks or longer.
- On fruits they survive for 1-5 days at room temperature and more than a week in refrigerator.
- E_l tor vibrios survive longer than the classical cholera vibrios.
- They survive in clean tap water for longer period than contraminated water.
- They are killed in gastric juice of normal acidity but may survive for 24 hours in achlorhydric gastric juice.

18. Ans. (c) *V. cholerae* toxin *Ref. Ananthanarayana 9/e, p 308; 8/e 306*

Already explained, refer Ans. 2

19. Ans. (c) and (d) Motility; and Binds to specific receptors *Ref. See below*

- To cause cholera, *Vibrio cholerae* must reach in small intestine where it produces cholera toxin.
- *Vibrio encounters* following barriers:

Barrier	Mechanism to invade
• Gastric acidity	– Large inoculum size (> 10^6 organism)
• Mucosal lining of small bowel	– Chemotaxis, motility and variety of protease
• Adhesion to epithelial cells	– Toxin corregulated pilus

20. Ans. (a) and (c) Food transmits and Healthy carrier *Ref. Ananthanarayan 8/e, p 306, 9/e, p 307; Park 21/e, p 208*

Mode of transmission of cholera:

- Fecally contaminated water ***(MC)***
- Contaminated food and drinks
- Direct contact.

Cholera vaccine	
Parenteral vaccine	***Oral vaccines***
Killed	Killed ***(wc/rbs)*** or live ***(CVD 103 HgR)***
Protective value 50%	Protective value 80% for live vaccine

So, no vaccine of cholera provide 90% protection.

Remember:

- ***V. cholerae* are killed within 30 minutes by heating at 56°C or within a few seconds by boiling.** Also killed by ***chlorination.***
- ***DOC*** for chemoprophylaxis is tetracycline. Alternative is doxycycline.
- Carriers in cholera includes preclinical or incubatory (1-5 days), convalenscent (2-3 weeks), contact or healthy (> 10 days) and chronic carrier.

Period of communicability	
Case:	7 days
Convalescent carrier:	2-3 weeks
Chronic carrier:	1 month - 10 year

21. Ans. (d) and (e) Neutrophilia and Occurrence of many cases in the same locality
Ref. Park 22/e, p 210; Harrison 17/e, p 970, 18/e, p 1292, 19/e, 1063

- *V. cholerae* cause ***noninflammatory (no WBC in stool)*** painless watery diarrhea.
- As secondary attack is high, many case occurs in same locality.

Lab features in Cholera:	– Elevated hematocrit – Mild neurophilic leukocytosis – Elevated BUN and creatinine – Normal sodium and chloride levels – Markedly reduced bicarbonate – Elevated anion gap

22. Ans. (c) Food can transmit the disease *Ref. Ananthanarayan 8/e, p 307, 9/e, p 307*

Already explained

23. **Ans. (b) 0:139** *Ref. Park 22/e, p 208*

0-139 posses novel 0-139 LPS which is responsible for its virulence. 0: 139 has replaced E_1 tor and is predominant cause of cholera in areas where it had appeared.

24. **Ans. (a, b) and (e) *Non-Vibrio cholerae* 01; Enterotoxigenic *E. coli*; *Vibrio cholerae*** *Ref. Harrison 17/e, p 814, 18/e, p 1088*

Causes of diarrhea with pus cells / RBC's in stool (inflammatory diarrhea or dysentery):

- *Shigella*
- *Salmonella*
- *Vibrio parahemolyticus*
- EIEC, EHEC
- *Yersinia*
- *Campylobacter*
- *Cl. difficle*

25. **Ans. (a and b) *Vibrio cholerae* .O1 and *Vibrio cholerae* O139** *Ref. Harrison 17/e, p 969, 18/e, p 1289, 19/e, p 1062*

Cholera now refers to disease caused by V. cholerae O1 or O139 i.e. the serogroups with epidemic potential.

26. **Ans. (a and d) V, cholerae; Camplyobacter jejuni** *Ref. Ananthanarayan 9/e, p 304*

Bacteria showing darting type motility

- Vibrio cholerae : Through single polar flagellum
- Campylobacter jejuni : Through single polar flagellum. Motility can be darting or tumbling type
- Sperillium minus

27. **Ans. (b, c, d) Voges Proskauer (VP) positive; Swarming; Cause sea borne auricular infections**

Ref. Ananthanarayan 9/e, p 312; Greenwood 18/e, p 320

V. alginolyticus

- High salt tolerant, ***VP positive***, sucrose fermenter halophilic vibrio that closely resembles V. parahemolyticus
- Forms large yellow colonies on TCBS
- It fails to grow on CLED agar but grows in presence of 10% NaCl. There is pronounced ***swarming*** on non selective solid media
- It is frequently found in ***sea fish***
- It has been associated with infections of eyes, ears and wounds in human beings exposed to sea water.

Chapter Review

1. **Which of the following about cholera is true:**
   a. Invasive [AI 90]
   b. Endotoxin is released
   c. Recent infections in India are of classical type
   d. Vibriocidal antibody titer measures prevalence
   [Ref. Ananthanarayan 8/e, p 311, 9/e, p 308]

2. **The function of B subunit of cholera toxin is:**
   a. ADP ribosylation of G protein [BHU 07]
   b. To bind GM1 ganglioside receptor
   c. To stabilize cholera toxin
   d. To increase cGMP
   [Ref. Ananthanarayan 9/e, p 308]

3. **7th pandemic of cholera is caused by:** [UP 07.] [DNB 2011]
   a. E_1 tor
   b. 0139 *V. cholerae*
   c. Classical *V. cholerae*
   d. *V. mimicus* *[Ref. Dr Arora 3/e, p 402]*

Pondemic	*Year*	*Causative vibrio*
1-6	1817-1923	Classical *V. cholerae*
7	1961	E_1 Tor
8	Not yet	0139 (proposed)

4. **Which of the following is not associated with *Vibrio cholerae*?** [APPG 08]
   a. Haemolytic uraemic syndrome
   b. Rice water stool
   c. Dehydration
   d. None *[Ref. Ananthanarayan 8/e, p 306, 9/e, p 307]*

5. **The cholera vibrio:** [UP 09]
   a. Is strongly anaerobic
   b. Grows best at 25°C
   c. Has marked tolerance of alkaline pH
   d. Grows over a wide range but best at a slight acid pH
   [Ref. Ananthanarayan 8/e, p 302, 9/e, p 304]

6. **Which of the following has shortest incubation period?**
   a. Plague b. Cholera [Delhi 2008]
   c. Measles d. Typhoid
   [Ref. Park 22/e p 209]

7. **Peritrichous flagella is seen in all except:** [Delhi 06, DNB-08]
   a. E. coli b. Salmonella
   c. Bacillus d. Vibrio cholerae
   [Ref. Ananthanarayan 8/e, p 302, 9/e, p 303]

8. **All of the following statements about cholera are true except:** [AI 97]
   a. O and H antigens measure carrier state
   b. Culture medium is TCBS agar
   c. Produces indole and reduces nitrate
   d. Synthesize neuraminidase *[Ref. Park 22/e, 209]*

   - **Enzymes produced by *Vibrio cholerae*:**
     - Neuraminidase
     - Elastase
     - Lipase
     - Mucinase
     - Chintinase.

   Mnemonic - Cute NELaM

9. **True about *Vibrio cholerae* is:** [SGPGI 03]
   a. Disease more common in woman
   b. Classical vibrio protect against development of 0.139 strain disease
   c. E_1-tor is more milder than classical
   d. Erythronycin is used in treatment
   [Ref. Ananthanarayan 8/e, p 308, 9/e, p 307, 308]

10. **All of the following statements are true for El- tor cholera except:** [AI 97]
    a. Infection is mild and asymptomatic
    b. They are resistant to polymyxin-B unit dikc
    c. Chronic carriers are common
    d. Secondary attacks rate is high in families
    [Ref. Ananthanarayan 8/e, 306, 9/e, p 308]

11. **True about cholera vibrios is:** [PGI 97]
    a. Can tolerate wide range of alkaline pH
    b. Nonmotile bacilli
    c. Cannot be grown in media
    d. NaCl stimulates growth *[Ref. Ananthanarayan 8/e, 302]*

    - Vibrio cholerae can grow in pH range 6.4-9.6 (Optimum - 8.2).
    - NaCl (0.5-1%) is required for optimal growth though high concentration (6% and above) is inhibitory.

12. **Halophillic vibrios includes all of the following except:**
    a. *V. vulnificus* b. *V. cholera* [SGPGI 07]
    c. *V. parahaemolyticus* d. *V. alginolyticus*
    [Ref. Ananthanarayan 8/e, p 316, 9/e, p 311]

13. **Which of the following statement is true about: *Vibrio cholerae*:** [AI 99]
    a. There is no natural reservoir
    b. Transported in alkaline peptone water medium
    c. Halophilic *[Ref. Ananthanarayan 8/e, 303, 9/e, p 304]*
    d. Oxidase negative

Answers 1. d. Vibriocidal 2. b. To bind GM1 ... 3. a. E_1 tor 4. a. Haemolytic ... 5. c. Has ...
6. b. Cholera 7. d. Vibrio ... 8. a. O and H .... 9. c. E_1-tor is more ... 10. d. Secondary...
11. a, d. Can tolerate and NaCl stimulates... 12. b. V. cholerae 13. b. Transported ...

NEET Pattern Questions

1. **Ogaura Inawa and Hikojima are the serotypes of:**
   a. Yersina
   b. Vibrio cholerae
   c. E Coli
   d. Salmonella typhi *[Ref. Ananthanarayan, 9/e, p 306]*

2. **Selective plating medium for V. cholerae is:**
   a. Carry-Blair medium
   b. TCBS agar
   c. VR medium
   d. MacConkey medium
   [Ref. Ananthanarayan, 9/e, p 309]

3. **True about cholerae includes all except:**
   a. Incubation period range from 1-5 days
   b. Produces isotonic diarrhea
   c. Cholera toxin plays principal role
   d. Antibodies 01 cholera provides protection against 0139 serotype also
   [Ref. Harrison, 18/e, p 1290]

> Clinical manifestations of disease caused by 0139 V. cholerae resemble that of 01 type V. cholerae. immunity to one however is not protective against the other.

4. **Which organism grows in alkaline pH?**
   a. Vibrio
   b. Klebsiella
   c. Pseudomonas
   d. E. coli

> Optimum pH for vibrio is 8.2 and it an grow in pH range of 6.4–9.6

5. **Invasive infection is caused by all except:**
   a. V. cholerae
   b. Neisseria
   c. Streptococci
   d. H. influenza

6. **Transport medium for cholera:**
   a. LJ medium
   b. Cary Blair medium
   c. MYPA medium
   d. Stewart medium *[Ref. Park 22/e, p210]*

7. **True about cholera:**
   a. Gram negative rod
   b. Associated with fever
   c. Causes painful watery diarrhea
   d. It is an achlorhydria which renders an individual susceptible to disease
   [Ref. Ananthanarayan, 9/e, p303, 307]

> **Transportation of cholera specimen:**
> - Stool should be transported in sterilized Mc. Cartney bottles containing alkaline peptone water on VR medium.
> - The specimen should be transported in alkaline peptone water on Carry blair medium if it is collected by a rectal swab.
> - It suitable plating media are available (e.g bile salt agar) at the bed side, the stools should be streaked on the media before forwarding to lab.

8. **Enrichment media for cholera:**
   a. VR medium
   b. TCBS medium
   c. Cary-Blari medium
   d. Alkaline peptone war
   [Ref. Ananthanarayan, 9/e, p 304]

9. **Optimal percentage of NaCl for V cholerae:**
   a. 1% b. 2%
   c. 3% d. 4%
   [Ref. Ananthanarayan, 9/e, p 304]

10. **Transmission of cholera is through:**
    a. Fecally contaminated food
    b. Fecally contaminated water
    c. Contaminated food by vomits of a case
    d. All of the above
    [Ref. Park, 22/e, p 209]

> Cases excrete around 10^7-10^{10} vibrios per ml of fecal fluid.
> Cariers excrete around 10^2-10^5 vibrios per gram of stool.

11. **True about vibrio parahemolyticus:**
    a. Polar flagella
    b. Non halophilic vibrus
    c. Non-capsulated
    d. Requires NaCl
    [Ref. Ananthanarayan, 9/e, p 311]

12. **True about vibrio vulnificus:**
    a. Causes diarrhea commonly
    b. Halophilic
    c. Drug of choice is penicillin
    d. Produces shiga toxin *[Ref. AA, 9/e, p 312]*

Answers 1. **b.** Vibrio cholerae 2. **b.** TCBS agar 3. **d** Antibodies... 4. **a.** Vibrio 5. **a.** V. cholerae
6. **b.** Cary Blair medium 7. **a.** Gram negative rod 8. **d.** Alkaline peptone... 9. **a.** 1% 10. **d.** All of the above
11. **d.** Requires NaCl 12. **b.** Halophilic

13. **Most halophillic vibrio:**
    a. V. cholerae
    b. V. vulnificus
    c. V. alginolyticus
    d. V. parahemolyticus *[Ref. Ananthanarayan, 9/e, p 309]*

14. **Most important step in cholera control:**
    a. Chemoprophylaxis
    b. Vaccination
    c. Early treatment
    d. Water sanitation *[Ref. Park, 22/e, p 211]*

15. **Non-Halophilic vibrio is:**
    a. V. cholerae
    b. V. parahaemolyticus
    c. V. alginolyticus
    d. V. fluvialis
    [Ref. Ananthanarayan, 9/e, p 304]

V. cholerae is halotolerant and high NaCl concentration (6% and above) are inhibitory

16. **True about El T or vibrio:**
    a. More SAR
    b. VP reaction (+)ve
    c. Low carrier rate
    d. More severe *[Ref. Ananthanarayan, 9/e, p 306]*

17. **Cholera toxin binds to which receptors in intestine:**
    a. Sphingosine through A subunit
    b. Sphingosine through B subunit
    c. GM1 gangliosides through A subunit
    d. GM1 gangliosides through B subunit
    [Ref. Ananthanarayan, 9/e, p 308]

18. **Bacteria with safety pin appearance:**
    a. H. influenzae
    b. Vibrio parahaemolyticus
    c. Vibrio vulnificus
    d. Salmonella paratyphi
    [Ref. Ananthanarayan, 9/e, p 311]

Answers **13.** **c.** V alginolyticus **14.** **d.** Water sanitation **15.** **a.** V. cholerae **16.** **b.** VP reaction (+)ve
17. **d.** GM1 gangliosides through B subunit **18.** **b.** Vibrio Parahaemolyticus

CHAPTER

17

Pseudomonas and Yersinia

PSEUDOMONAS

Gram (–)ve aerobic motile bacilli with polar flagella.
***Pseudomonas aeruginosa* is the *MC* human pathogen in this group.**

> I
> **Pseudomonas:** Non capsulate, non-fermentative, non sporing, Aerobic Gr (–)ve, pigment producing, motile (two flagella) bacteria
> ***Selective media:*** Cetrimide agar

P. aeruginosa = P. Pyocyanea

- *Non-sporing, obligate aerobic bacilli* which is differentiated from enteric gram (–)ve bacilli by its ***ability to oxidise indophenol and inability to ferment lactose.***
- ***Noncapsulated*** but many strains have mucoid slime layer of alginate particularly in patient of cystic fibrosis.
- Part of normal intestinal flora. *...Jawetz 27/e, p 245*

Note: Can grow anaerobically if nitrate is available as a terminal electron acceptor.

Culture and Growth characteristic

- Grows well at 37-42°C on ordinary media.
- ***Growth at 42°C** helps differentiate it from other Pseudomonas species.*
- ***Selective media*** – Cetrimide agar
- Pigment
  a. ***Pyocyanin*** – Bluish green pigment produce *only by P. aeruginsoa.* It inhibits growth of many other bacteria.
  b. ***Fluorescein*** – Greenish yellow. Produce by all species of *Pseudomonas.*
  c. ***Pyorubin***
  d. ***Pyomelanin***] – *Some strains of P. aeroginosa*

> I
> **Fluoroscent pigment:** pyoverdin
> **Red pigment:** Pyorubin
> **Black pigment:** Pyomelanin

Classification

- *On the basis of difference of lipopolysaccharide.*
- Restriction endonuclease typing with pulsed gel electrophoresis is most reliable method.
- Used for epidemiological purpose.

Pathogenicity and Resistance

- ***P. aeroginosa*** is pathogenic only when inculated into areas devoid of normal defences's e.g. when mucous membrane is disrupted, patient is catheterized, neutropenia is there.
- ***MC*** and ***most serious*** cause of infection in burns.
- ***MC infection*** outside hospital is suppurative otitis media.
- Causative agent of Shanghai fever.
- ***Produce Blue pus*** with characteristic fruity odour.
- ***Pathognomonic*** skin lesion are called as *ecthyma gangrenosum,* which occur singly or in small number on the perineum, buttocks and extremities.
- Resistant to common antiseptic and disinfectant such as dettol. Contrary it may grow profusely in bottle of these antiseptic. Resistant to quaternary ammonium compounds, chloroxylenol.

Virulence factors

• Pili or fimbriae	• Lipopolysaccharide or endotoxin
• Mucoid exopolysaccharide/alginate	• Alkaline protease
• Elastase	• Hemolysin
• Exotoxin (A, S, T, U, Y)	• Heat stable phospholipase

> I
> Exotoxin of Psudomonas inhibits protein synthesis

- ***Exotoxin A*** – As, ↓ protein synthesis via ADP ribosylation and thereby inactivation of EF-2.
- ***Exotoxin S*** – Ribosylation of GTP binding protein, disruption of cellular actin cytoskeleton.
- Exoenzyme U is a phospholipase.
- Exoenzyme Y is an adenylyl cyclase.

Remember: Extracellular virulence factors exhibits Quorum Sensing (= cell to cell signaling system). P. aeroginasa can be typed by lipopolysaccharide immunophenotype and by pyocin (bacteriocin susceptibility) *... Jawetz 27/e, p 247*

Sterilization –Killed at 55°C in 1 hour; Sensitive to acids, β glutaraldehyde, silver salts and strong phenolic disinfectants

Treatment

Antimicrobials effective against Pseudomonas are:

Penicillin	*Cephalosporins*	*Aminoglycoside*	*Quinolones*
Piperacillin/tazobactam	Ceftazidime	Tobramycin	Ciprofloxacin
Tazobactam	Cefoperazone	Gentamicin	Levofloxacin
Ticarcillin/Clavulanate	Cefepime	Amikacin	Trovafloxin
Mezlocillin			

Other Agent: Polymyxin B. Colistin, *Monobactams* - Aztreonam

- **Drug of Choices: Aminoglycoside + Penicillin except:**
  a. **In UTI** – Ciprofloxacin
  b. **In CNS infection** – Ceftozidime ± Aminoglycoside.
  c. **In Malignant external otitis** – Cephalosporin or carbapenem or ciprofloxacin.

I

B. pseudomallei
- Causative agent of meliodesis
- Motile, oxidase (+)ve.
- Bipolar safety pin appearance

Burkholderia Pseudomallei [Pseudomonas Pseudomallei]

- ***Causative agent of*** 'Melioidosis'.
- Resembles *Ps. mallei* but differs in being motile.
- Organism is oxidase positive and motile but does not produce diffusible pigments.
- *MC* manifestation of melioidosis: Acute pulmonary infection.
- May cause hemoptysis resembling TB.
- Another common manifestation is acutely localizead skin infection with ulceration or abscess that is associated with nodular lymphangitis and regional lymphadenitis.
- Latency and reactivation may occur as bacillus can survive intracellulary in reticuloendothelial system.
- Human infection occurs commonly through skin abrasion or by inhalation.
- **Diagnosis:** – ***Typical bipolar safety pin appearance*** of bacillus in exudates on microscopy.
  – Confirmed by culture or ≥ 4 fold rise in antibody.
- **Treatment:** – ***Ceftazidime or carbpenems*** is *DOC.*

Burkholderia Mallei (Pseudomonas Mallei)

- ***Causative agent of*** 'Glanders', a disease of equine.
- Non-motile with bipolar stained organism giving safety pin appearance.
- ***On potato:*** characteristic amber, honey like growth appears, becoming greenish yellow resembling Ps. aeruginosa
- It is agent of biological Warfare and terrorism, classified as 'category B biological agent'.
- In human, it cause acute localized suppurative infection, acute pulmonary infection; acute septicemic infection and chronic suppurative infection.
- *It induces* ***'straus reaction'.***
- Human infection is usually ***occupational.***
- **Diagnosis:** – *Mallein test* which is analogous to tuberculin test.
  – *Molecular methods for rapid identification – 16s rRNA gene sequencing. Also distinguish it from B. pseudomallei.*
- **Treatment:** – Same as **melioidosis.**

I

B. mallei
- Causative agent of glanders
- Non-motile
- Both mallei and pseudomallei are potential bioterrorism agent

PASTEURELLAE

- Group consists of gram-negative, short, pleomorphic bacilli that are primary pathogen of rodents.
- It is divided into 3 genus:
  - a *Yersinia:* – Includes plague bacillus (*y.pestis*); *Y. pseudotuberculosis* (primary pathogen of rodents); *Y. enterocolitica.*
    – It is assigned in the family Enterobacteriaceae.
  - b. *Pasteurella:* – Includes *P. multocida* (non-motile, oxidase positive).
  - c. *Francisella:* – Includes F. Tularensis.

Yersinia Pestis = Plague Bacillus

Morphology

- Nonmotile, nonsporing, microaerophilic, biochemically unreactive, pleomorphic bacilli/coccobacillus.
- Characteristic bipolar (safety pin) appearance, with Wayson's stain/Giemsa/methylene.
- It is noncapsulated but at ≥ 30°C it produces envelop ***(= F1 antigen)*** – a virulence factor that serves as the principal immunodiagnostic marker of infection.
- *Serotypes do not exist.*
- Central Asia or Himalayas is believed to be the original home of plague.
- The last pandemic was started in Hong Kong in 1894 and was caused by Y. pestis var orientalis.

I

Yersinia
- Non-motile
- Non-sporing
- Microaerophillic
- Safety pin appearance on Wayson's stain
- Cause plague

Cultural Characteristics

- *Optimum* growth occurs at ***27°C and pH 7.2*** (unlike most pathogens which usually grow at 37°C).
- Shows 'Stalactite growth' in ghee broth.

Remember: Growth is *fastest* at 30°C and envelops develop best at 37°C. It produce coagulase at 28°C but not at 35°C. ..... *Jawetz 25/e, p 257*

Resistance: Killed at 55°C in 5 min and by 0.5% phenol in 15 min.

Biochemical reactions:

- Based on fermentation of glycerol and reduction of nitrate, it is divided into 3 varieties.
- Catalase and aesculin positive; Urease and oxidase negative.

I

Non-capsulated but at ≥ 30°C it produces envelop

Pathogenesis

- From the site of flea bite ***(xenopsylla cheopis)*** it is carried to regional lymph nodes (=bubo) via lymphatic channel.
- Mononuclear phagocytes play role in dissemination of infection to distant sites (secondary pneumonia and septicemia).
- **Primary pneumonia** results by droplet infection of plague patient.
- **Primary septicemic plague** consist of sepsis in absence of bubo while secondary septicemic plague is complication of bubonic or pneumonic plague.
- Heat stable somatic antigen complex of Y. pestis has endotoxic activity which contribute to terminal toxima. Heat labile portion resist phagocytosis.

Clinical Features

- Characterized by rapid onset of fever and other systemic manifestations of Gram –ve bacterial infections.
- It is of 3 types:
  - a. **Bubonic Plague**: – I.P 2 - 7 days
    – *MC* type of plague and is almost always caused by ***bite of infected flea.***
    – *MC* site of bubo, inguinal region >axillary, cervical.

... *Harrison 18/e, p 1307*

I
MC site of bubo: Axillary node

- Distinguished from lymphadenitis by its rapid onset, extreme tenderness, accompanying signs of toxemia and absence of cellulitis or obvious ascending lymphadenitis.
- DIC is common and may lead to gangrene.
- *Y. pestis can also be cultured from blood of most bubonic plague patient.*

b. Septicemic plague: (black death)
- Most fatal, often present with GI symptoms, DIC, multiorgan failure.
- I P 2 - 7 days

c. Pneumonic plague:
- ***Most infectious***, type of plague with I P of 1 - 3 days. Can cause:
  (i) Primary pneumonia: Cyanosis is very prominent, with bloody mucoid sputum.
  (ii) Secondary pneumonia: Diffuse ***interstitial*** pneumonia, less infectious.

Diagnosis:
- Demonstrating the bicilli in fluid from buboes or local skin lesion
- Blood culture
- PCR is rapid & less hazardous.

I
- Bubonic plague is the most common type of plague
- Pneunonic plague is the most fatal type
- Plague can be transmitted from rat to rat, rat to human, human to human.

Treatment:
- Streptomycin is drug of choice

Prevention :
- ***Prophylaxis of choice*** – Tetracycline.
- Recombinant vaccine that use F_1 and V antigen is available.

FRANCISELLA OR PASTEURELLA OR BRUCELLA TULARENSIS

- Capsulated, nonmotile, intracellular parasite which grows in special media such as Francis blood dextrose cystine agar.
- It resembles *Mycoplasma*.
- It cause ***tularemia***, a disease of rabbits and other rodents which is transmitted by ticks.
- In human it presents as local ulceration with lymphadenitis, typhoid like fever with glandular enlargement or influenza like respiratory infection.
- **Treatment:** Streptomycin is *DOC* for adults and children.

YERSINOSIS

- ***It denotes infection with Yersiniae other than Y. pestis*** (i.e. by enterocolitica and pseudotuberculosis).
- They are motile at 25°C but non-motile at 37°C.
- They show antigenic cross-reaction with *Y. pestis, Vibrio, Salmonella, Brucella.*
- For culture *'cold enrichment'* is done.

Y. pseudotuberculosis
- Most human infection occur by serotype 01.
- ***Commonest presentation is abdominal pain & fever***
- Associated with Far-Eastern scarlet like fever (a childhood illness with rash, arthralgia and toxic shock) similar illness is called as Izumi fever in Japan.

Y. enterocolitca
- Most human infections occur by serotypes 03, 08, 09.
- It causes gastroenteritis or enterocolitis; Mesenteric adenitis or terminal ileitis; system disease with bacteremia; erythema nodosum, reactive arthritis (in HLA - B 27).

Multiple Choice Questions

1. **The mode of infection of *Pasteurella multocida*?**
   a. Endogenous infection **[AI 09]**
   b. Animal bites or scratches
   c. Human beings
   d. Aerosols and dust

2. **A young boy had a flea bite while working in a wheat grain godown. After 5 days he developed fever and had axillary lymphadenopathy. A smear was sent to the laboratory to perform a specific staining. Which one of the following staining method would help in the identification of the suspected pathogen?** **[AI 06]**
   a. Albert staining
   b. Ziehl-Neelsen staining
   c. McFadyean's staining
   d. Wayson staining

3. **Which one of the following drugs is an antipseudomonal penicillin?** **[AI 06]**
   a. Cephalexin b. Cloxacillin
   c. Piperacillin d. Dicloxacillin

4. **The following statements are true regarding melioidosis except:** **[AI 05]**
   a. It is caused by *Burkholderia mallei*
   b. The agent is a gram-negative aerobic bacteria
   c. Bipolar staining of etiological agent is seen with methylene blue
   d. The most common form of meliodosis is pulmonary infection

5. **An organism grown on agar shows green colored colonies; likely organism is:** **[AI 01]**
   a. *Staphylococcus* b. *E. coli*
   c. *Pseudomonas* d. *Peptostreptococcus*

6. **A girl from Shimla presented with fever, malaise and axillary/inguinal lymphadenopathy and organism shows stalactite growth on culture. Which of the following is the causative organism?** **[AIIMS 08]**
   a. *Yersinia pestis* b. *Francisella*
   c. *Burkholderia pseudomallei* d. *Pasteurella*

7. **Which species of *Pseudomonas* is the most common cause of intravenous catheter related infection?**
   a. *Pseudomonas cepacia* **[AIIMS 08]**
   b. *P. aeruginosa*
   c. *P. maltophilia*
   d. *Burkholderia pseudomallei*

8. **All of the following statement about plague are wrong except:** **[AIIMS 04]**
   a. Domestic rat is main reservoir
   b. Bubonic is the most common variety
   c. The causative bacillus can survive upto 10 years in the soil of rodent burrows
   d. Incubation period for pneumonic plague is one to two weeks

9. **A 50-year-old chronic alcoholic male agriculture worker presented with high grade fever of one week with spells of chills and rigor. Examination of the respiratory system reveals bilateral crepitation with scattered rhonchi. Multiple subcutaneous nodules were found on extensor surface of left forearm and left leg. Direct microscopy of the pus aspirate from the nodules reveals plenty of grams (–)ve bacilli with bipolar staining. Culture reveals distinct rough corrugated grey white colonies on blood agar. The organisms were motile and oxidase-positive. The most likely diagnosis is:**
   a. Plague b. Melioidosis **[AIIMS 03]**
   c. Bartonellosis d. Actinomycosis

10. **The drug of choice for chemoprophylaxis in contacts of a patient of pneumonic plague is:** **[AIIMS 02]**
    a. Penicillin b. Rifampicin
    c. Erythromycin d. Tetracycline

11. **True about *Y. pestis*:** **[PGI 06, 03]**
    a. Gram +ve b. Gram -ve
    c. Motile d. Non-motile
    e. It is coccobacilli

12. **True statement(s) about *Y. pestis* is/are:** **[PGI 04]**
    a. Gram-positive
    b. Non-motile
    c. Benzyl penicillin is given in prophylaxis
    d. Patients are kept isolated till 48 hr of treatment
    e. Repeated blood culture is diagnostic

13. **A farmer from Himachal Pradesh present with inguinal lymphadenopathy. On examination multiple small ulcers are seen in leg. Which of the following stain would you prefer for bipolar staining of etiologic agents of this case?** **[AIIMS 2010]**
    a. KOH b. Gram stain
    c. Wayson's d. Nigrosin

14. **A farmer presents to the emergency department with painful inguinal lymphadenopathy and history of fever and flu like symptoms. Clinical examination reveals on ulcer in the leg. Which of the following stain should be used to detect suspected bipolar stained organism.**
    a. Alberts stain b. Ziehl Neelsen stain **[AI 2011]**
    c. Wayson's stain d. McFayden's stain

15. **Farmer presents with the features of high fever, painful inguinal lymphadenopathy, vomiting diarrhea and hypotension. Which stain will help in the diagnosis?** **[AIIMS NOV 2012[**
    a. Neisser stain b. Wayson's stain
    c. Albert's stain d. Mcfadyean's stain

Explanations and References with Illustrative Answers

1. **Ans (b) Animal bites or scratches** *Ref. Ananthanarayan 8/e, p 326, 9/e, p 325*

Pasteurella multocida

- Group of bacteria similar to *Yersinia* often carried in the upper respiratory tract of variety of animals such as dogs, cats, cattle. It may cause hemorrhagic septicemia in these animals.
- *P. multocida* is ***nonmotile***, Gram-negative bacilus, resembling *Yersinia*, but differing in being oxidase-positive, ***producing indole*** and *failing to grow on MacConkey agar.*
- Human infection is rare but may occur following animal bites or scratches.
- Clinical manifestation includes local suppuration, meningitis (following bite in head) respiratory tract infection.
- Tetracycline and streptomycin are drug of choice.

2. **Ans. (d) Wayson staining** *Ref. Park 22/e, p 267-268; Jawetz 25/e, p 258; Ananthanarayan 8/e, p 323, 9/e, p 321-323*

"It is typical clinical presentation of bubonic plague."

PLAGUE:

- It is a zoonosis.
- Causative agent is Yersinia pestis which is:
  - Gram-negative, non-motile, coccobacilli
  - Exhibits bipolar staining with ***Wayson's stain/giemsa stain/methylene blue, Wright Stain***
  - Plague bacilli can survive and indeed multiply in the soil of rodent burrows where microclimate and other conditions are favourable.
- Reservoir:
  - ***Wild rodents*** (e.g. field mice) ***are natural reservoirs*** of plague.
  - *In India wild rodent,* ***Tatera indica*** *has been incriminated as main reservoir,* not the domestic rat.
- Source of infection:
  - ***Infected rodents*** and ***fleas*** and ***case of pneumonic plague (not bubonic plague).***
- Immunity:
  - After recovery there is relative immunity.
- Vector:
  - Commonest vector is rat flea, (X-cheopis). Infected flea may live upto 4 year.
  - Human **infection** is ***most frequently*** contracted from bite of infected flea.
  - ***Basic cycle*** in epidemic bubonic plague is: Commensal rats → rat fleas → man
- **Incubation period:**
  - Bubonic plague - 2 to 7 days
  - Septicaemic plague - 2 to 7 days
  - Pneumonic plague - 1 to 3 days
- Clinical Presentation:
  - ***MC type*** of human ***plague*** is ***bubonic plague*** characterized by enlarged tender lymph nodes (Bubos).
  - MC site of bubo is inguinal, axillary and cervical nodes.
  - **Pneumonic plague** – Rare variety but most infectious variety of plague.
  - **Septicaemic plague** – Rare variety.
- Diagnosis:
  - ***Specimens*** - Blood for culture.
    - Aspirates of enlarged lymph nodes for smear and culture.
  - ***Smears*** - Stain with Giemsa and specific immunofluorescent stains.
    - Prime face diagnosis is by examination of smears which show characteristic ***bipolar appearance with Wayson's stain.***
  - ***Culture*** - Blood culture are often positive in 24 hours. Show stalactite growth
    - Definite identification of culture is best done by immunofluorescence.
  - ***Serology*** - Antibodies to F. 1 antigen may be detected by passive hemagglutination.
- **Treatment:** – **DOC** – Streptomycin (Alternative tetracycline, chloramphenicol)
- **Chemoprophylaxis:** – **DOC** – Tetracycline (Alternative sulfonamide)

Remember: Flea bone disease – Endemic typhus – Chiggerosis – Hymenoplepis diminata

Other Options

Stain	Organism
Albert's	*C. diphtheria*
Ziehl-Neelsen	Acid fast organism
Mc Fadyean's	*B. anthrax*

3. **Ans. (c) Piperacillin** *Ref. Harrison 18/e, p 1269; 16/e, p 894*

Antimicrobial agents active against Pseudomonas aeruginosa		
Antipseudomonal penicillins	***Antipseudomonal cephalosporins***	***Carbapenems/Other agents***
• Piperacillin	• Ceftazidime	• Imipenem/cilastatin
• Mezlocillin	• Cefoperazone	• Meropenem
• Ticarcillin	• Cefepime	• Polymyxin B
• Ticarcillin/clavulanate		• Colistin
Monobactams	**Aminoglycosides**	**Fluoroquinolones**
• Aztreonam	• Tobramycin	• Ciprofloxacin
	• Gentamicin	• Levofloxacin
	• Amikacin	

4. **Ans. (a) It is caused by *Burkholderia mallei*** *Ref. Harrison 17/e, p 955, 18/e, p 1273; Jawetz 27/e, p 248*

Meliodoisis

- Caused by *Burkhoderia pseudomallei (Pseudomonas pseudomallei).*
- It is free living small, motile (differentiating feature from pseudo. mallei) *aerobic gram-negative* bacillary saprophyte normally found in soily ponds and rice paddies.
- It grows at 42°C and oxidise glucose, lactose and is *oxidase positive.*
- It forms colonies that vary from mucoid and smooth to rough and wrinkled and in colour from cream to orange.
- *MC* form of melioidosis is *Acute pulmonary* infection.
- Acute pulmonary infection vary from mild bronchitis to extensive necrotizing pneumonia.
- Chronic pulmonary infection mimics TB.
- It also cause acute, localized skin infection with ulceration or abscess that is associated with nodular lymphangitis and regional lymphadenitis.
- Also cause suppurative parotitis particularly in children.
- Progression of disease is more common in chronic debilitated patient (DM, chronic renal disease, alcoholics).

Diagnosis

- Considered in patient present with acute lower respiratory tract illness, parotitis, lymphadenitis or unusual skin or subcutaneous lesion or chest X-ray suggest TB (upper lobe infiltrate) in absence of tubercle bacilli in sputum.
- Gram's stain of appropriate specimen will show small Gram-negative bacilli; **bipolar regularly staining** (safety pin appearance) is seen by Wright's stain or methylene blue stain.
- **Positive culture is diagnostic.**
- Positive serologic test is evidence of past infection.
- X-ray - upper lobe infiltrate occasionally with thin walled cavities.

Treatment:

- Ceftazidime or carbepenems are DOC.

5. **Ans. (c) Pseudomonas** *Ref. Ananthanarayan 8/e, p 314, 9/e, p 314*

Pseudomonas

Aerobic, nonsporing gram-negative, motile bacilli, forms many pigments:

a. **Pyocyanin:** – Bluish green pigment, produced **only by** Ps. aeruginosa
b. **Fluorescin (Pyoverdin):** – Greenish yellow pigment which oxidise in old culture to yellowish brown pigment
c. **Pyrubin:** – Red
d. **Pyomelanin:** – Brown

Other Pigment forming bacteria	
• *S. aureus*	– Golden yellow pigment
• *B. melanogenicus*	– Black pigment
• *Rhodococcus*	– Red pigment
• *Nocardia*	– Yellow to red pigment
• *Peptostreptococcus*	
• *Photo and Scotochromogen*	– Yellow orange pigment

6. **Ans. (a)** ***Yersenia pestis*** *Ref. Ananthonarayan 8/e, p 323, 9/e, p 372*

In India presently there are four foci of plague

1. Near kolar in Tamil Nadu
2. Beed Latur belt of Maharashtra
3. Rhoru near Shimla in Himachal Pradesh
4. Small pocket in Uttaranchal

So, in a girl of Shimla with clinical features of plague and organism showing stalactite growth can be none other than Y. pestis.

7. **Ans. (b)** ***P. aeroginosa*** *See below*

Etiology of bacteremia associated with IV catheters	
• Staph. epidermidis	85%
• S. aureus	7%
• Candida albicans	3%
• Enterococcus	2%
• Others – *E. coli* – *Kleibsiella* – *P. aeroginosa* – Viridans streptococci	3%

8. **Ans. (b) Bubonic is the most common variety** *Ref. Ananthanarayan 8/e, p 323, 9/e, p 322*

Already explained, refer Ans. 2

9. **Ans. (b) Melioidosis** *Ref. Harrison 17/e, p 955, 18/e, p 1272; Jawetz 27/e, p 248*

- Actinomycosis is ruled out as it is gram-positive bacilli.
- Plague is ruled out as it is gram-negative nonmotile coccobacilli.
- Bartonellosis is ruled out as it does not exhibits bipolar staining.
- Bacteria showing bipolar staining = safety pin appearance are:
  - Calymmatobacterium granulomatis – Yersinia and Pasteurella – H. ducreyi
  - V. parahemolyticus – Ps. pseudomallei

10. **Ans. (d) Tetracycline** *Ref. Park 18/e, p 237; Ananthanarayan 8/e, p 324, 9/e, p 324*

Already explained

11. **Ans. (b, d) and (e) Gram –ve, Non-motile; and It is coccobacilli** *Ref. Ananthanarayan 8/e, p 320, 9/e, p 319*

Yersinia is gram –ve, nonmotile cocobacilli.

12. **Ans. (b) and (e) Non-motile; and Repeated blood culture is diagnostic** *Ref. Park 21/e, p 271; Ananthanarayan 8/e, p 320, 7/e, p 329*

Already explained

Note: Drug of choice for chemoprophylaxis is tetracycline

13. **Ans. (c) Wayson's** *Ref. Jawetz 27/e 276; Ananthanarayan 8/e 323, 9/e 323*

This is a case of bubonic plague

Why?

- The geographical Area - Himachal Pradesh which is one of the foci of plague in India

Other are:

- Kolar at the trijunction of AP, TN, Karnataka
- Beed - Latur belt in Maharashtra
- Small pocket in Uttaranchal

Clinical Presentation of Patient

- Enlarged tender inguinal lymphnodes is commonest manifestation of bubonic plague.
- Multiple small ulcer, represent flea bites

Now coming to question

- Plague is caused by Yersina pestis a gram-negative short bacilli
- In smear stained with Wayson, Giemsa or methylene blue, it shows bipolar staining ***(safety pin appearance)*** with the two ends densely stained and the central area clear.
- The bacilli characteristically show pleomorphism which gets enhanced in media containing 3% NaCl. It is non- motile, non-sporing and non-acid fast.

14. **Ans. (c) i.e. Waysons stains** *Ref. Ananthanarayan 8/e, p 323, 9/e 323; Jawetz 27/e, p 276*

Aready explained

15. **Ans. (b) i.e. Waysons stains** *Ref. Ananthanarayan 8/e, p 323, 9/e 323; Jawetz 27/e, p 276*

Aready explained

Chapter Review

1. **Which of the following does not cause food born infection?** [Delhi 08]
   a. *Salmonella* b. *B. cereus*
   c. *Pseudomonas* d. *E. coli*
   [Ref. Ananthanarayana 8/e, p 316, 9/e 315]

2. **Blue pus found in infection:** [RJ 05]
   a. *Staph* b. *Streptococcus*
   c. *Pseudomonas* d. *Mycoplasma*
   [Ref. Ananthanarayana 8/e, p 316, 9/e 316]

3. **Glanders is caused by:** [DNB 2012]
   a. *Protozoa* b. *Virus*
   c. *Fungi* d. *Bacteria*
   [Ref. Greenwood 18/e, 303]

4. **Pyocyanin is formed by:** [UP 2012]
   a. *Yersinia*
   b. *Pseudomonas*
   c. *Burkholderia*
   d. *Pasteurella* *[Ref. Greenwood 18/e, 299]*

Answers 1. c. *Pseudomonas* 2. c. *Pseudomonas* 3. d. *Bacteria* 4. b. *Pseudomonas*

NEET Pattern Questions

1. **Pseudomonas toxin acts by:**
   a. ↑cAMP b. ↓cAMP
   c. ↓Protein synthesis d. ↓cGMP
   [Ref. Ananthanarayan, 9/e, p 316]

2. **A diabetic person presents with multiple abscess in leg. Microscopic examination of pus shows gram (-)ve bacilli. On staining with methylene blue bacteria shows bipolar staining. The most likely causative agent is:**
   a. B. pseudomallei b. Y. Pestis
   c. Pseudomonas mallei d. Botromycosis
   [Ref. Ananthanarayan, 9/e, p 317]

3. **In India last outbreak of plague occurred in which state:**
   a. Gujarat b. Maharashtra
   c. Himachal Pradesh d. MP
   [Ref. Ananthanarayan, 9/e, p 322]

> Last outbreak occurred in February 2002 near Shimla

4. **Pseudomonal infection, not cleaned by:**
   a. Dettol b. Hypochloritic
   c. Chlorine d. Betadine
   [Ref. Ananthanarayan, 9/e, p 315]

> Pseudomonas is resistant to common antiseptics and disinfectants such as quaternary ammonium compounds, chloroxylenol and hexachlorophane. Indeed P. aeroginosa can grow in dettol or cetrimide selective medium.

5. **Ecthyma gangrenosum is caused by:**
   a. Pseudomonas b. Streptococcus
   c. Staphylcoccus d. H. influenza

> **Ecthyma gangrenosum**
> - Occurs exclusively in markedly neutropenic patient or AIDS patient.
> - The disease is characterized by small or large, painful, — maculopopular lesions, which are initially pink then darken to purple and finally to black.
> - These lesions are due to vascular invasion and are teeming with bacteria.

6. **What is not true about yersiniosis:**
   a. Zoonosis
   b. Caused by Y pestis
   c. By Yersinia enterocolitica
   d. By Yersinia pseudotuberculosis
   [Ref. Ananthanarayan, 9/e, p 324]

7. **Selective media for Pseudomonas:**
   a. EMJH medium b. PALCAM agar
   c. PLET medium d. Cetrimide agar
   [Ref. Ananthanarayan, 9/e, p 315]

> Cetrimide prevents the growth of alternate flora and also entrances the production of Pseudomonas pigments such as pyocyanin and fluorescein.

8. **An organism grown on agar shows green coloured colonies, likely organism is:**
   a. Staphylococcus b. Streptococcus
   c. Pseudomonas d. E. coli
   [Ref. Ananthanarayan, 9/e, p 315]

9. **Pseudomonas exotoxin inhibits protein synthesis:**
   a. RNA polymerase b. EF-2
   c. Transpeptidase d. Reverse transcriptase
   [Ref. Internet, 9/e, p 315]

> Exotoxin A, the main virulence factor of betadomonas exerts its pathogenic effect by inhibition of protein synthesis via ADP-ribosylation and there by inactivation of elongation factor 2.

10. **Bacteria that can grow even in the presence of antiseptic:**
    a. Staphylococcus b. Streptococcus
    c. E. coli d. Pseudomonas
    [Ref. Ananthanarayan, 9/e, p 315]

11. **Which of the following is non-motile:**
    a. Pseudomonas aeruginosa
    b. Burkholderia mallei
    c. Burkholderia pseudomallei
    d. None of the above *[Ref. Ananthanarayan, 9/e, p 317]*

12. **Pneumonic plague is spread by:**
    a. Bite of infected flae
    b. Direct contact with infected tissue
    c. Ingestion of contaminated food
    d. Droplet infection *[Ref. Ananthanarayan, 9/e, p 322]*

13. **Izumi fever is caused by:**
    a. Pseudomonas aeruginosa
    b. Burkholderia mallei
    c. Yersinia pseudotuberculosis
    d. Pasteurella multocida *[Ref. Harrison, 19/e, p 1077]*

14. **A patient in ICU and on ventilator develops cough with fever. The gram-staining on microscopy will show:**
    a. Gram negative cocci b. Gram negative bacilli
    c. Gram positive bacilli d. Gram variable organism

Answers 1. **c.** ↓ Protein synthesis 2. **a.** B. pseudomallei 3. **c.** Himachal Pradesh 4. **a.** Dettol
5. **a.** Pseudomonas 6. **b.** Caused by Y pestis 7. **d.** Cetrimide agar 8. **c.** Pseudomonas
9. b. EF-2 10. **d.** Pseudomonas 11. **b.** Burkholderia mallei 12. **d.** Droplet infection
13. **c.** Yersinia pseudotuberculosis 14. **b.** Gram negative bacilli

CHAPTER

18

Hemophilus, Bordetella and Brucella

I

Hemophilus:
- Non motile, Gram (–)ve bacilli
- H. influenzae and H. hemolyticus require factors X and V for growth
- Flides agar is best for isolation of H. influenzae
- H. influenzae shows satellitism cause meningitis, epiglottitis, cellulitis

HEMOPHILUS

- Genus comprised of nonmotile, nonsporing, oxidase positive gram-negative bacilli, characterized by requirement of one or both of two accessory growth factors (X and V) in blood. These requirement are used to identify the bacteria.
- Important species are *H. influenza, H. aegypticus* and *H. ducreyi.*
- Both *H. influenzae* and *H. haemolyticus* have identical growth (factors X and V) requirement. So *H. hemolyticus is distinguished from H. influenzae by hemolysis on horse blood agar.*

H. INFLUENZAE = PFEIFFER'S BACILLUS

Morphology

- Capsulated Coccobacilli shows pleomorphism.
- Stained by ***Loeffler's methylene blue*** or Dilute carbol fuchsin.
- On the basis of indole production, urease and ornithine decarboxylase activity, it is divided into eight biotypes.

Culture

- ***Flides agar*** is ***best for*** primary isolation.
- On ***Levinthal's medium***—capsulated strain shows distinctive iridescence.
- Require ***both X factor*** (heat stable hemin) and V factor (heat labile coenzyme present in RBC) so heated or boiled blood agar (Chocolate agar) is superior to plain agar. X factor is not required for anaerobic growth.
- Shows *'Satellitism'* (dependence on V factor) when *S. aureus* is streaked across blood agar. Staphylococus on sheep blood agar cause the release of NAD which favours the growth of H. influenzae.

I

Invasive disease is more common.

Antigenic Properties

- **There are three major surface antigen -** the capsular polysaccharide; the outer membrane protein (OMP) and Lipo-oligosaccharide.
- Major antigenic determinant is **capsular polysaccharide** based on which, it is typed into six capsular types **a to f** while noncapsulated strains are nontypable.
- Most isolates from **acute invasive** infections belong **to 'b'.**
- Type b capsule has unique structure containing pentose sugar (ribose and ribitol) in the form of Polyribosyl ribitol phosphate (PRP) instead of hexoses and hexosamines as in other 5 serotypes. The capsule of type b H. influenzae is the major virulance factor.
- Hib PRP is used in vaccine.
- *H. influenza* is first free living organism whose *complete genome is sequenced.*

Feature	Type b Strains	Nontypable Strains
Capsule	Ribosyl-ribitol phosphate	Noncapsulated
Pathogenesis	Invasive infections due to hematogenous spread	Mucosal infections due to contiguous spread
Age	2 months–3 years	Adults
Clinical manifestations	Meningitis and invasive infections in incompletely immunized infants and children	Otitis media in infants and children; lower respiratory tract infections in adults with chronic bronchitis and pneumonia, puerperal sepsis
Vaccine	Highly effective conjugate vaccines	None available; under development

I

Many non-encapsulated H influence are the part of normal microbiota of the upper respiratory tract.

- **Serotyping of *H. influenzae* is done by agglutination or quelling reaction**

... DR Arora 3/e, p 431

Clinical Features

Meningitis: **Most** frequently by **biotype - I** of type **b** strain
- Occur in children (<2 years of age) due to absence of PRP antibodies.
- ***MC* Complication** of its meningitis - *Subdural effusion*

Epiglottitis: - Particularly affects 2-7 years age group.
Cellulitis: - Seen in young children. The most common location is on the head or neck.
Pneumonia: - Particularly in infants.
- Hib is more likely to involve pleura *...Harrison 19/e, 1011*

Diagnosis:

Viability of H. influenzae declines with time particularly at 4°C. So they should be transported rapidly.

Meningitis: - CSF gram's stain and culture on IsoVitale X-enriched chocolate agar *... Jawetz 27/e, 265*
- If culture negative - **Detection of PRP.**

Respiratory tract infection: - Suggested by predominance of gram-negative coccobacilli among abundant polymorphonucelar leukocytes in sputum.

Treatment : *DOC* ceftriaxone or cefotaxime (also in other invasive infection).

Vaccination : Hib conjugate vaccine:
- 1st dose : 2 months
- Rest of primary series : 2-6 months
- Booster dose : 12-15 months.

Remember: No vaccine is available for nontypable H. influenza.

I

H. egyptius – Conjunctivitis
– Brazilian purpuric fever
H. ducreyi : Chancroid

I

Non typable H. influenzae is the most common bacterial cause of exacerbation of COPD.

I

Resistant to ampicillin d/t production of β lactamase and altered penicillin binding protein

HAEMOPHILUS DUCREYI

- Bipolar stained *(safety pin)* bacilli arranged in group or whorls or parallel chains (school of fish or rail-road track appearance).
- Causative agent of *Chancroid or soft sore*—STD characterized by tender, non-indurated irregular genital ulceration and inguinal adenitis, i.e painful penile ulcer with inguinal lymphadenopathy.
- Primary isolation is difficult. It can be grown on fresh clotted rabbit blood. Smears made after 24–48 hours of incubation show tangled chains of bacilli.

Treatment:
- Single oral dose of azithromycin.
- Alternative ciprofloxacin or erythromycin.

Note: An unrelated Gram negative rod calymmatobacterium granulomatis cause similar STD called as granuloma inguinale or donovanosis *...Harrison 19/e,880*

Clinical Features of penile ulcer					
Feature	**Syphilis**	**Herpes**	**Chancroid**	**Lymphogranuloma venereum**	**Donovanosis**
Incubation period	9–90 days	2–7 days	1–14 days	3 days–6 weeks	1–4 weeks (up to 6 months)
Early primary lesions	Papule	Vesicle	Pustule	Papule, pustule, or vesicle	Papule
No. of lesions	Usually one	Multiple	Usually multiple, may coalesce	Usually one; often not detected, despite lymphadenopathy	Variable
Diameter	5–15 mm	1–2 mm	Variable	2–10 mm	Variable
Edges	Sharply demarcated, elevated, round, or oval	Erythematous	Undermined ragged, irregular	Elevated, round, or oval	Elevated, irregular
Depth	Superficial or deep	Superficial	Excavated	Superficial or deep	Elevated
Induration	Firm	None	Soft	Occasionally firm	Firm
Pain	Uncommon	Tender	Very tender	Variable	Uncommon
Lymphadenopathy	Firm, nontender, bilateral	Firm, tender, often bilateral	Tender, may suppurate, usually unilateral	Tender, may suppurate, loculated, usually unilateral	None; pseudobubboes

HAEMOPHILUS AEGYPTIUS

Identical to non-capsulated H. influenzae, therefore now named as H. influenzae biotype aegypticus. Cause highly contagious form of conjunctivitis *(PINK EYE)* and Brazilian purpuric fever *(BPF)*.

BPF: Conjunctivitis proceed to fulminant septicemia in infants and children with high fatality.

BORDETELLA

- Genus consist of gram-negative, strict aerobic coccobacilli which grows only on complex media.
- Its two important members:
  a. ***B. pertussis*** – Causative agent of pertussis (whooping cough = 100 day fever).
  b. ***B. parapertussis*** – Silent copy of pertussis toxin gene causing milder form of pertussis.

I

Bordetella
- Gr (–)ve, aerobic coccobacilli
- **Enriched media:**
  - Regan Towe
  - Bordet-Gengou glycerine potato
- B. pertussis is the most fastideus bordetella
- Pertussis toxin acts by activating G protein
- Acellular vaccine, containing PT and filamentous hemaglutinin is the preferred vaccine

Morphology

- *B. Pertussis* is pleomorphic, nonmotile, nonsporing, ***capsulated, fimbriated*** coccobacilli, which show Bipolar metachromatic granules on staining with toluidine blue. Most fastidious of all bordetellae
- Obligate human parasite

Culture

- Grows on enriched media like *Regan Towe or Bordet-Gengou glycerine potato blood agar*, forming colonies with bisected pearls or Mercury drops or Aluminium paint appearance.
- Culture films has **Thumb print** appearance.
- Charcoal containing media (as for legionella) is preferred.
- Blood is required to neutralize the inhibitory materials formed during bacterial growth.

Virulent Factors

- **Pertussis toxin**
  - Most important virulence factor
  - ***PT is a exotoxin*** protein consist of B - (binding unit) and A (active unit) having ADP ribosylating activity of G protein ***(like of cholera toxin)***
  - *B parapertussis* do not express the gene coding for pertussis toxin.
  - Pertusis toxin also serves as adhesin, lymphocytosis producting factor, histamine sensitizer and islet activating protein.
- ***Filamentous hemagglutinin (FHA) secreted protein.***
  - Both PT and FHA hemagglutinin promotes secondary infection by coating *H. influenza* and pneumococci so that they bind. This is known as *'Piracy of Adhesins'*.
- **Tracheal cytotoxin**
  - It is responsible for destruction of the ciliated respiratory epithelial cells. Tracheal cytotoxin inhibits DNA synthesis in ciliated cells
- Surface adhesins (pertactin, Fimbriae), Adenylate cyclase, hemolysin, heat labile toxin LPS endotoxin, pertactin agglutinogens are other virulence factor.
- Impairment of host defence by B pertussis is mediated by pertusis toxin and adenylate cyclase toxin. *... Harrison 19/e 1022*

I

Whooping cough is non-invasive infection of respiratory mucosa.

Pathogenesis

- Infection is initiated by attachment of the organism to the ciliated epithelial cells of the nasopharynx, attachment is mediated by surface adhesions.
- Infection is limited to respiratory mucosa only, local cellular invasion with intracellular persistence (systemic dissemination not occurs). Systemic manifestation is due to toxin.
- Both cellular and humoral immunity are important.
- First defence is by antibody which prevents attachments of bacteria.
- Neurological manifestations are due to hypoxia.

Clinical features

- *Incubation period:* 7–14 days.
- *It has 3 stages:*

a. *Catarrhal stage:* Maximum infectivity
b. *Paroxysmal stage:*
   - Post-tussive vomiting is frequent with mucus plug occassionally at end of episode.
   - Vomiting with cough is the best predictor of diagnosis of pertussis.
   - Episodes are often worsening at night and interfere with sleep.
   - Most complications occur during paroxysmal stage.
   - Paroxysm is precipitated by noise, eating and physical contact
c. *Convalescent stage*

I

Purpose of antibiotic therapy is to eradicate the infecting bacteria from the nasopharynx. Therapy doesn't alter the clinical course unless given early in catarrhal phase.

Complications

Subconjunctival hemorrhages, abdominal and inguinal hernia, pneumothorax, petechiae, weight loss, apnea, pneumonia, seizures, encephalopathy.

Diagnosis

i. *Best specimen* is obtained by *nasopharyngeal aspiration* ..... *Harrison 19/e, p 1023*
ii. Gold standard – Culture of nasopharyngeal secretion
iii. Absolute lymphocytosis without ↑ in ESR
iv. *Most sensitive is PCR*
v. Serology – If symptoms > 4 weeks.

Treatment

- *DOC* – Macrolide (Erythromycin, Clarithromycin, Azithromycin)
- Alternative – Cotrimoxazole
- β agonist, Glucocorticoids and cough suppressants are *not effective.*

Prevention

- *Chemoprophylaxis*
  - For household contact of cases.
  - Erythromycin is *DOC.*
- *Immunization – main stay* of preventions is active immunization.
  Two types of vaccine are available:
  a. *Whole cell vaccine:*
     - Associated with many adverse effects and may also cause – encephalopathy, sudden infant death syndrome, and autism.
     - It is contraindicated in individual ≥ 7 year age.
  b. *Acellular vaccine:*
     - Less reactogenic and is recommended for routine immunization.
     - All variety of acellular vaccine contain pertussis toxoid and filamentous hemagglutinin.
     - It contains pertussis toxoid.
     - Two component vaccine are more effective than monocomponent, since addition of pertactin increase efficacy.
     - Protection against pertussis by vaccine correlated best with the production of antibody to pertactin, fimbriae, and pertussis toxin.

BRUCELLA

- Gram-negative, nonmotile, noncapsulated, non-sporing, strict aerobic, intracellular coccobacilli.
- Major virulence factor: LPS which possess endotoxin activity.
- Brucella is agent of biological warfare.
- Form both caseating and non-caseating granuloma.
- Resist intracellular killing by suppression of myeloperoxidase – hydrogen peroxide-halide system and production of superoxide dismutase.
- Organism are sensitive to sunlight, heat, ionizing radiation but are resistant to freezing and drying.

I

Brucella
- Gr (–)ve, intracellular coccobacilli that grows on strict aerobic condition
- B. abortis requires 5-10% CO_2
- B. melitensis is the most common and most virulent cause of brucellosis
- Diagnosis requires castaneda method of blood culture.

I

Souce of infection: Sheep, goats, camel
Mode of infection: Usually occupational.
Most serious manifestation: Pancarditis.

Culture

- B. abortus require 5-10% CO_2 for growth, where as other three species can grow in air.
- Fresh specimens from animal or human source are usually inoculated on trypticase soy agar or blood culture media: Optimum temperature is 37°C. Erythritol has a stimulating effect on the growth of brucellae.
- In vivo brucellae behave as facultative intracellular parasite. *... Harrison 18/e, p 1296*

Classification

- On the basis of CO_2 requirements, H_2S production, sensitivity to dyes, phage lysis, oxidative metabolic tests four species are identified–B. melitensis, B. abortus are major species.

Clinical features

I

MC and most virulent cause of Brucellosis: B. melitensis

- It cause Brucellosis, a zoonotic diseases, also known as mediterranean fever or Malta or undulant fever.
- *Source:* Sheep, goats, camels.
- ***Route of transmission:*** Occupational exposure, ingestion of untreated milk or milk products, raw meat (blood); inhalation; transplacental; during breastfeeding and during sexual activity.
- *MC* and ***most virulent*** cause of brucellosis: *B. melitensis.*
- *MC* **symptoms** are fever, chills, diaphoresis, headaches.
  Pancarditis, Sleep disturbances, lymphadenopathy, Hepatosplenomegaly, GBS syndrome, abortion or IUD during pregnancy are other important findings.
- *MC* focal feature is musculoskeletal pain.
- **Death is** usually a consequence of *cardiac involvement.*
- Immunity is mainly cell-mediated.

Diagnosis

1. Most definitive method – Blood culture
   - **Castaneda** method of blood culture is employed.
   - Preferred media are serum dextrose agar; trypticase soy agar.
2. *PCR: More sensitive* and more quicker than blood culture.
3. *Serological test:*
   a. Acute infection – Standard agglutination (SAT) test done
      – Shows prozone phenomenon
      – SAT also +ve in cholera, tularemia, yersinia, and immunization.
   b. Chronic infection – Complement fixation test.
   c. For diagnosing animal infection – Rapid ***plate agglutination, rose Bengal card*** and ***milk ring test done.***

Treatment

- TB must always be excluded.
- ***Gold standard*** treatment in adults: ***Streptomycin + doxycycline.***
  ... Harrison 17/e, p 976, 18/e, p 1299
- Alternative rifampin + doxycycline.
- In children, pregnant women or who cannot tolerate tetracycline – Cotrimoxazole given.

Multiple Choice Questions

Hemophilus

1 (a). All the following are true about *H. influenzae* except: [AIIMS 03]
- a. It can be part of normal flora of some persons
- b. The serotyping is based on bacterial outer membrane protein
- c. It requires Hemin and NAD for growth in culture medium
- d. Type b is responsible for invasive disease

1 (b). In a child admitted with H. Influenza meningitis, Cefotaxime was started instead of ampicillin. Which of these is the likely reason for this? [AIIMS 15]
- a. H. influenzae stains known to produce Beta lactamase
- b. H. influenzae stains known to have altered penicillin binding protein
- c. More easier to give
- d. It is cheap

2. A 2 years old child is brought to the emergency with history of fever and vomiting. On examination he has neck rigidity. CSF examination shows polymorphs more than 200 /ml; protein 100 mg/dl and glucose 10 mg/dl. the Gram's stain shows the presence of Gram-negative coccobacilli. The culture shows of bacteria only on chocolate. Agar and not on blood agar. The causative agent is: [AIIMS 02]
- a. *Neisseria meningitidis*
- b. *Haemophilus influenzae*
- c. *Branhamella catarrhalis*
- d. *Legionella pneumophila*

3. Disease caused by *Hemophilus* species:
- a. Chancroid [PGI June 09]
- b. Influenza
- c. Acute epiglottitis
- d. Brain abscess
- e. Brazilian purpuric fever

4. False about H. influenzae: [AIIMS Nov 10]
- a. It needs X and V factor for growth
- b. Protein capsule plays an important role in pathogenecity
- c. It is a rare cause of meningitis in the first 2 months of life
- d. Most common invasive manifestation is meningitis

5 (a). True about H. influenza: [PGI May 2013]
- a. Also called as Pfeiffer's bacilli
- b. In acute infections capsulated strains are often isolated
- c. Gram negative motile bacilli
- d. Easily stainable
- e. VP test positive

5 (b). Non-typable H. influenzae can cause all except:
- a. Meningitis
- b. Otitis media
- c. Puerperal infection
- d. Pneumonia

Bordetella

6. The following are true for *Bordetella pertussis* except: [AI 03]
- a. It is a strict human pathogen
- b. It can be cultured from the patient during catarrhal stage
- c. It leads to invasion of the respiratory mucosa
- d. Infection can be prevented by a acellular vaccine

7. The usual incubation period of pertussis is:
- a. 7-14 days [AIIMS 05]
- b. 3-5 days
- c. 21-25 days
- d. Less than 3 days

8. True regarding pertussis vaccine is: [AIIMS 00]
- a. 95% of vaccinated are protected
- b. Erythromycin should be given to contacts
- c. Neuroparalytic complication is seen in 1 in 15000
- d. Leukocytosis is diagnostic

9. Mechanism of action of pertussis toxin is all except:
- a. Act by ADP ribosylation of GI subunit [AIIMS 08]
- b. Increase in calcium
- c. Act by decreasing GTP

10. In which of the following organism does the capsule does not act as a virulence factor? [AI 2011]
- a. H. influenzae
- b. Strep pneumoniae
- c. N. meningitidis
- d. Bordetella pertusis

11. A 7-month-old, partially immunized child presented with cough ending in characteristic whoop. Which of the following is considered the best type of specimen to isolate the organism and confirm the diagnosis [AI 11, AIIMS May 11]
- a. Nasopharyngeal swab
- b. Cough plate culture
- c. Tracheal-aspirates
- d. Sputum

12. **Acellular pertussis vaccine contains:** [AIIMS 2011]
    a. Pertactin, flagillary hemagglutinin, cytotoxin, endotoxin
    b. Pertactin, flagillary hemagglutinin, fimbriae, endotoxin
    c. Pertactin cytotoxin, fimbriae
    d. Flagillary hemagglutinin, pertussis toxin, fimbriae

13. **All are true statement regarding pertussis, except:**
    a. Secondary attack rate averages 90% in unimmunized contacts [PGI 2012]
    b. Incubation period is around 14 days
    c. Erythromycin is the drug of choice
    d. Can affect people of any age
    e. Main source of infection is chronic carriers

Brucella

14. **A veterinary doctor had pyrexia of unknown origin. His blood culture in special laboratory media was positive for gram-negative short bacilli which was oxidase positive. Which one of the following is the likely organism grown in culture?** [AI 06]
    a. *Pasturella spp.* b. *Francisella spp.*
    c. *Bartonella spp.* d. *Brucella spp.*

15. **A farmer presenting with fever off and on for the past 4 years was diagnosed to be suffering from chronic Brucellosis. All of the following serological tests would be helpful in the diagnosis at this state except:** [AI 04]
    a. Standard agglutination test
    b. 2-mercapto-ethanol test
    c. Complement fixation test
    d. Coomb's test

16. **Malta fever is caused by:** [AIIMS 08]
    a. *Legionella*
    b. *Borrelia burgdorferi*
    c. *Brucella melitensis*
    d. *Pseudomonas*

17. **Brucellosis can be transmitted by all of the following modes, except:** [AIIMS 07, 06]
    a. Contact with infected placenta
    b. Ingestion of raw vegetables from infected farms
    c. Person to person transmission
    d. Inhalation of infected dust or aerosol

18. **Brucella is transmitted by all the following means except:** [AIIMS 06]
    a. Through placenta of animals
    b. Person to person transmission
    c. Aerosol
    d. Eating uncooked food

19. **A farmer rearing sheep, presented with complaints of fever and weakness for the last one month. There is generalised lymphadenopathy. There was also associated hepatomegaly Biopsy of liver showed non-caseating granuloma. There are most likely due to infection with:**
    a. *Yersinia pesitis* [AIIMS 00]
    b. *Brucella canis*
    c. *Francisella tularensis*
    d. *Brucella melitensis*

20. **Regarding Brucellosis, all of these are true except:**
    a. Man to man transmission [PGI 02]
    b. A zoonosis
    c. Blood cultures used in diagnosis
    d. Transmitted via animal products

21. **All are true about brucella except:** [AIIMS May 11]
    a. B. abortus is capnophilic
    b. Transmitted by aerosol can occur occasionally
    c. Paesturisation destroys it
    d. 2 ME is used to detect IgA

22. **Brucella melitensis is commonly found in (animal):**
    a. Pig
    b. Camel
    c. Sheep
    d. Goat
    e. Reindeer [PGI 2011]

Explanations and References with Illustrative Answers

1 (a). Ans. (b) The serotyping is based on bacterial outer membrane protein *Ref. Ananthanarayan 8/e, p 331, 9/e, p 335*

H. influenzae strains have been classified on the basis of capsular polysaccharide *(not on the basis of outer membrane protein.)*

Important features of *H. influenzae (Pfeiffer's bacillus)*

- Gram (–)ve coccobacilli
- Require ***Factor V*** (nicotinamide nucleotide) and ***Factor X*** (hemin) for growth. So grow well on chocolate agar as factor V is free in chocolate agar (Growth is poor in blood agar).
- **Antigenic structure:**
  - a. Capsular antigen – Basis of classification
    - Most isolates of acute invasive infection belongs to type b.
  - b. Bacterial outermembrane protein
  - c. Lipooligosaccharide
- Noncapsulated strains of *H. influenzae* can colonize the upper respiratory tract of childrens and adults.

1 (b). Ans. (a) H. influenzae stains known to produce Beta lactamase *Ref. Harrison 17/e, p 924*

Approximately 20-35% of nontypable strains of hemophilus produce β-lactamase. In addition to β-lactamase alteration of penicillin binding proteins is a second mechanism of ampicillin resistance has been detected in isolates of H. influenzae.

2. Ans. (b) *H. influenzae* *Ref. Ananthanarayan 8/e, p 329, 9/e, p 327*

See the morphology of asked bacteria, you will know the answer.

H. influenzae	– Gram-negative coccobacilli
N. meningitidis	– Gram-negative cocci
Legionella	– Gram-negative coccobacilli
B. catarrhalis	– Gram-negative cocci

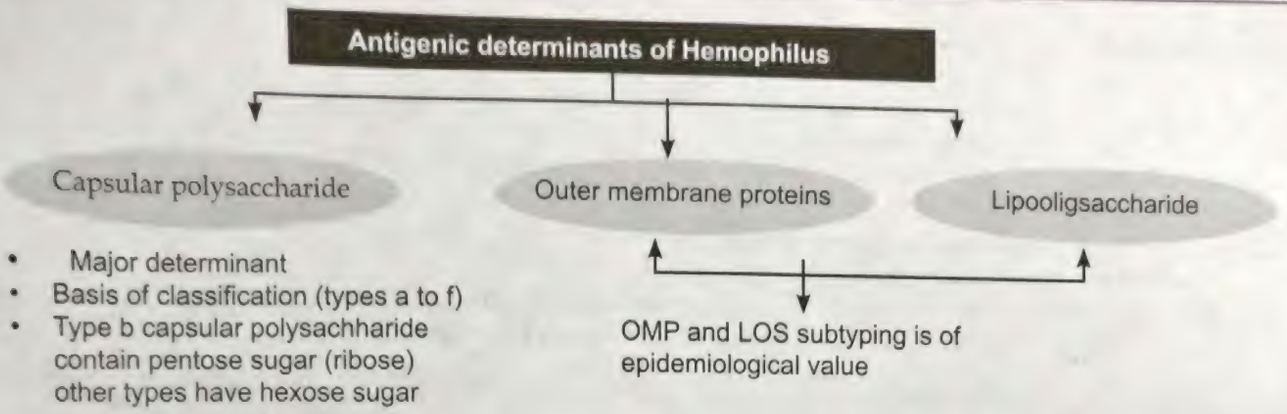
3. Ans. (a), (c) and (e) Chancroid, Acute epiglottitis, Brazilian purpuric fever *Ref. Ananthanarayan 8/e, p 331-332, 9/e, p 327*

Disease Caused by *Haemophilus*	
Species	***Disease***
H. influenzae	– Meningites – Epiglottitis – Pneumonia – Bronchitis, arthritis, pericarditis, – Endocarditis
H. aegypticus	– Brazilian purpuric fever – Conjuctivitis
H. ducreyi	– Chancroid or soft sore

Note: Influenza is a viral disease.

4. Ans. (b) Protein capsule plays an important.... *Ref. Ananthanarayan 8/e, p 331, 9/e, p 328*

Capsule is made up of polysaccharide (not protein)



5a. Ans. (a, b) Also called as Pfeiffer's bacilli, In acute infections capsulated strains are often isolated *Ref. Ananthanarayan 9/e, p 327*

H. influenzae was identified by Pfeiffer who isolated H. influenzae in patients of influenzae.

5b. Ans. (a) Meningitis *Ref. Harrison 18/e, p 1228-1229, 19/e, p 1011*

Strains of Hemophilus which lacks polysaccharide capsule are referred as Non-typable Strains

- Non-typable H. influenza is the commonest bacterial cause of exacerbations of COPD.
- They also cause community acquired pneumonia, otitis media (3rd most common cause after pneumococci, and Moraxella catarrhalis), puerperal sepsis, neonatal bacteraemia, sinusitis.

6. Ans. (d) Infection can be prevented by an acellular vaccine *Ref. Ananthanarayan 8/e, p 338, 9/e, p 337; Harrison 17/e, p 936*

"All acellular vaccine current available contain pertussis toxoid hence they only prevent disease not infection."

..... Harrison 17/e, p 936

Bordetella pertussis

- *Bordetella pertussis* is an obligate human parasite.
- **Source** of infection is case of pertussis.
- **Carriers** and **Subclinical** infection are not found.
- *Infection is limited to respiratory tract*; in advance conditions leads to desquamation of alveolar epithelium (invasion of respiratory mucosa).
- Bacilli do not invade bloodstream.
- **Diagnosis:**
  - *Culture* of *nasopharyngeal secretions* remains the gold standard of diagnosis.
  - *Best specimen* is collected by *nasopharyngeal aspiration.*
  - Culture is positive in catarrhal and early paroxysmal stage.
  - Media used is Bordet-Gengou or Regan-Lowe.

Remember: Regan-Lowe charcoal medium—Transport media.

7. Ans. (a) 7-14 days *Ref. Park 22/e, p 154*

Incubation period of pertussis is usually 7 to 14 days, but never exceed 3 weeks.

Important points about pertussis:

- Caused by *Pertussis*
- Source of infection is case, there is no evidence of subclinical infection
- Pertussis is most infectious in catarrhal stage
- Infective period extends from a week after exposure to about 3 weeks after the onset of paroxysmal stage
- Secondary attack rate is about 90% in unimmunized contacts.

8. Ans. (b) Erythromycin should be given to contacts *Ref. Park 22/e, p 155*

Those known to have in contact with whooping cough should be given prophylactic erythromycin or ampicillin for 10 days.

Pertussis vaccine

1. **Killed whole cell vaccine:**
   - Given in the form of DPT
   - Protection 70-90%

Adverse reaction:
- Neurologic complication 1:170000
- Convulsions
- Prolonged screaming
- Hyporesponsible state.

Contraindication:
- Epilepsy, convulsion or other CNS disorder.

2. **Acellular vaccine:**
   - Contain pertussis toxoid and filamentous hemagglutinin.
   - Same efficacy but less reactogenic
   - Two component (which contain pertactin and pertussis toxoid. are more effective than monocomponent acellular vaccine. The further addition of fimbriae appears to enhance protective efficacy. ..... *Harrison 17/e, p 936*

Note: In pertusis lymphocytosis (not leukocytosis) is diagnostic.

9. **Ans (a) Acts by ADP ribosylation of GI subunit** *Ref. Jawetz 27/e, p 266; Chakraborty 2/e, p 387*

Pertusis toxin (Lymphocytosis promoting factor)
- Molecular weight of 117000 and is made-up of 6 polypeptide chain
- It has got two subunits A (Active) and B. A unit has ADP ribosylating activity of G protein resulting in increased adenylate cyclase activity which in turn leads to an elevation in CAMP with disturbance to normal cell function such as excessive insulin secretion, histamine sensitization.

Remember: – Tracheal cytotoxin is responsible for cough paroxysm.
– Pertussis toxin is responsible for lymphocystosis

10. **Ans. (d) Bordetella pertussis** *Ref. Harrison 17/e, p 933, 18/e, p 1241; Jawetz 27/e, 267*

Antigenic constituents and Virulence factor of Bordetella
- Pertussis toxin (most important)
- Dermonecrotic toxin
- Adenylate cyclase toxin
- Filamentous hemagglutinin (a component of cell wall)
- Tracheal cytotoxin
- Lipo oligosoccharide
- Fimbriae
- Pertactin (an outer membrane protein)

Note: All virulance factors of Bordetella are regulated by a single locus on chromosome. This locus has two Bordetella virulence genes bvg A and bvg S. 'bvg' S responds to environmental signal whereas bvg A is a transcriptional activator of virulence gene.

11. **Ans. (a) Nasopharyngeal swab** *Ref. Harrison 17/e 935, 18/e, p 1244*

Culture of nasopharyngeal secretion is the gold standard for diagnosis. The best specimen is collected by nasopharyngeal aspirations. An alternative to the aspirate is a dacron or rayon nasopharyngeal swab.

Note: PCR, due to its quick result, is replacing culture in many laboratories.

12. **Ans. (d) Flagillary ...** *Ref. Harrison 18/e 1245*

All acellular pertussis vaccines contain pertussis toxoid and filamentous hemagglutinin. One acellular vaccine also contain pertactin and another contains pertactin plus two types of fimbriae.

Phase III studies suggest that addition of pertactin and fimbriae increase the efficacy of vaccine.

13. **Ans. (e) i.e. Main source of infection.....** *Ref. Ananthanarayan 8/e 335-339, 9/e p 335; Park 21st/e 153*

"Source of pertussis infection is always a case, a chronic carrier state does not exists and there is no evidence that infection is ever subclinical."

For full details see previous answers.

14. **Ans. (d) Brucella *spp.*** *Ref. Ananthanarayan 8/e, p 342, 9/e, p 340*

Oxidase positive gram-negative organism producing pyrexia of unknown origin in veterinary doctor is pointing towards Brucella (coccobacilli or short rods).
- **Brucella is the causative agent of Brucellosis** *(Malta fever, undulant fever).*

- **Other options:**

Pasturella spp. (P. multocida)

- It is also short bacilli and oxidase positive, but it can grow over normal media and does not present with pyrexia of unknown origin. It is nonmotile and produces indole.
- Manifestation of pasturella: Local suppuration, Meningitis, Respiratory tract infection.

Francisella (Casuative agent of Tularemia):

- It is also short bacilli with fastidious growth requirement. But it is oxidase negative.

Remember:

- Brucella is oxidase and catalase positive except *B. neotomae* and *B. ovis*.
- **Risk factors for Brucellosis** (Zoonotic disease).
  - *Occupational*—Farmers, shepherds, Veterinarians, Goats herds, Slaughter-house workers.
  - *Domestic*—family members of individual in animal husbandry.
  - *Laboratory workers*—involved in handing cultures.
  - Travellers and Urban dwellers.
- Brucella can grow over normal media but growth is improved by addition of serum or liver extract.

15. Ans. (a) Standard agglutination test *Ref. Ananthanarayan 8/e, 344, 9/e, p 342-343; Harrison 17/e, p 975, 18/e, p 1299; Jawetz 27/e, p 270*

Diagnosis of Brucellosis

1. **Culture:**
   - Blood culture is the most definitive method.
   - ***Castaneda method*** of blood culture is recommended.
2. **Serology**
   a. ***Standard agglutination (tube agglutination) test (SAT)*** *is performed most often. It identifies mainly the IgM antibody.*
      - *Titre tends to decline after acute phase of the illness* so not helpful in chronic brucellosis.
      - *Positive agglutination test may be produced by cholera, Tularemia or* Yersinia *infection or immunization.*
      - *Cholera induced agglutinins may be differentiated by agglutination absorption test and also as they are removed by treatment with* 2-mercapto ethanol.

   b. ***Blocking or nonagglutinating antibodies*** *—Most reliable method for obviating the blocking effect and detecting the incomplete antibodies is antiglobulin (Coomb's) test.*

   c. ***Complement fixation test*** *—Detects both IgG and IgM so it is more useful in chronic cases.*

   d. ***ELISA*** *—Sesitive, specific and can detect IgM and IgG antibody separately so useful for differentiating acute and chronic infections.*

   e. ***PCR*** *—To detect bacteremia, to predict relapse and to exclude chronic brucellosis. More sensitive and quicker than blood culture.*

   f. *Brucellacapt: Rapid immunocapture agglutination method based on the combs test that detects non agglutinating IgG and IgA antibodies. It has a high sensitivity and specificity.*
3. **Hypersensitivity test - Not useful** in diagnosing acute infections.

16. Ans. (c) *Brucella melitensis* *Ref. Ananthanarayana 8/e, p 342, 9/e, p 340*

Already explained

17. Ans. (c) Person to person transmission *Ref. Park 22/e, p 265*

Brucellosis is transmitted from infected animal to man and ***there is no evidence of transmission from man to man.***

Mode of Transmission of Brucella		
Contact infection (MC)	***Food borne infection***	***Airborne infection***
– Direct contact with infected tissue, blood, urine, etc.	– Ingestion of raw milk or dairy products	– From aerosols in cowshed and slaughter house
– Mostly occupational	– Water contaminated with excreta of infected animal	

18. Ans. (b) Person to person transmission *Ref. Park 22/e, p 265*

Already explained, refer just above answer.

19. Ans. (d) *Brucella melitensis* *Ref. Harrison 17/e, p 974, 18/e, p 1297*

History of contact with sheep and clinical features of fever, weakness, lymphadenopathy with noncaseating granuloma signifies Brucellosis.

- *Brucella melitensis* is most pathogenic and most common cause of brucellosis and usually infect sheep, goats and camels.
- *Brucella canis* usually infect dogs
- ***Brucella abortus*** – Cattle or buffalo
- ***Brucella suis*** – swine
- *No* man to man transmission occurs.

Clinical features of Brucellosis

- Fever with profuse sweats, especially at night. If untreated fever follows undulating pattern, i.e. fever → afebrile period → fever.
- Fever is associated with musculoskeletal symptoms, i.e. pain.
- Osteomyelitis commonly in lumbar and low thoracic vertebrae.
- Septic arthritis ***(MC knee)***
- Neurologic involvement with depression and lethargy
- Endocarditis *(MC in aortic valve)*

20. Ans. (a) Man to man transmission *Ref. Park 22/e, p 265; Harrison 17/e, p 973, 18/e, p 1296*

Already explained

21. Ans. (d) 2 ME is used to detect IgA *Ref. Ananthanarayan 8/e, p 341, 9/e p 343*

2ME (2-Mercaptoethonol test) detect IgM and IgG

Serological tests for Brucellosis

- Standard Agglutination test
- Rose Bengal test
- Enzyme Immune Assay (detects IgM, IgG, IgA)

Culture Characteristics of Brucella

- Strict aerobs, B. abortus is capnophillic
- Optimum temperature 37°C
- Can grow on normal media, growth is improved by the addition of serum or liver extract. Erythritol has a specific stimulating effect on the growth of brucellae.

Resistance

- They survive in soil and manure for several weeks; remain viable for 10 days in refrigerated milk, 1 month in ice cream and for > 4 months in butter
- They are sensitive to 1%, destroyed by heat at 60°C in 10 minutes. They are killed by pasteurisation. They are sensitive to sunlight and acid.

22. Ans. (b, c and d) i.e. Camel, Sheep, Goat *Ref. Park 22/e, p 265*

- Animals that commonly acts as source of human brucellosis are: Goats, sheep, cattle, buffaloes, and swine
- In some part of world infection may also come from dogs, reindeer caribou, camels and yalks
- Most important vehicle of infection is raw milk. Milk products, meat from infected animal and raw vegetables or water supply contaminated by feces or urine of infected animal may also be responsible.

Chapter Review

1. **Milk ring test is for:** **[MP 2009]**
   a. Brucellosis
   b. Bacteroides
   c. Tuberculosis
   d. *Salmonellosis*

   [Ref. Ananthanarayan 8/e, p 345, 9/e, p 343]

   Milk ring test is used to detect brucella infection in cattle. In this test a sample of whole milk is mixed with a drop of stained brucella antigen and incubated in water bath at 70°C for 40-50 min. If antibodies are present, bacilli are agglutinated and form a blue ring at the top; leaving the milk unstained. Other tests are:

   - Rapid plate agglutination test
   - Rose Bengal card test

2. ***Haemophilus ducreyi* is the causative agent is:** **[Kar 01]**
   a. Soft sore
   b. Hard chancre
   c. Urethritis
   d. Granuloma inguinale

   [Ref. Ananthanarayan 8/e, p 333, 9/e, p 331]

3. ***Haemophilus ducreyi* causes:** **[Kolkata 04]**
   a. Lymphogranuloma venereum
   b. Chancroid
   c. Syphilis
   d. Psittacosis

   [Ref. Ananthanarayan 8/e, p 333, 9/e, p 331]

4. **All are true statement regarding pertussis except:** **[DNB 2013]**
   a. Secondary attack rate averages 90% in unimmunized contacts
   b. Incubation period is around 14 days
   c. Erythromycin is during of choice
   d. Can affect people of any age
   e. Main source of infection is chronic carriers

   [Ref. Ananthanarayan 9/e p 335]

5. **All of the following are virulence factors of pertussis, except:** **[DNB 08]**
   a. *Tracheal cytotoxin* *[Ref. Jawetz 25/e, p 218]*
   b. *Pertussis toxin*
   c. *Capsule*
   d. *Pertactin*

Answers 1. a. Brucellosis 2. a. Soft sore 3. b. Chancroid 4. e. Main source of infection... 5. c. Capsule

NEET Pattern Questions

1. **True about H. influenza:**
   a. Grown on sheep blood agar and CO_2
   b. It is not capsulated
   c. Invasive strain is most common
   d. Gram positive *[Ref. Harrison, 18/e, p 1229]*

2. **Mouse is used for pathogenicity testing in:**
   a. M. Tuberculosis
   b. C. diptheriae
   c. Bordetella pertussis
   d. Brucella *[Ref. Ananthanarayan, 8/e, p 337]*

3. **Whooping cough is caused by:**
   a. B. pertussis
   b. H. influenzae
   c. Pneumococcus
   d. Meningococcus
   [Ref. Ananthanarayan, 8/e, p 341, 9/e p 335]

4. **Which does not has a known animal reservoir**
   a. Brucella melitensis
   b. Bordettela pertussis
   c. Pasturella multocida
   d. Francisella tularensis
   [Ref. Ananthanarayan, 9/e p 333, 335]

Bordetella pertussis infect only human. A related species B. bronchiseplica had been isolated from dogs with broncho-pneumonia.

5. **Chancroid is caused by:**
   a. H. ducreyi
   b. T. pallidum
   c. Gonococcus
   d. HSV *[Ref. Ananthanarayan, 9/e p 331]*

6. **True about H. influenza:**
   a. Grown on sheep blood ager and CO_2
   b. It is not capsulated
   c. Invasive strain is most common
   d. Gram positive

Hib strains cause systemic disease by invasion and hanatogenous spread from the respiratory tract to distant site such as meninges, bones and joints. The type b polysaccharide capsule is an important virulence factor and imparts bacterium the ability to avoid opsonisation and cause systemic disease.

7. **Agar media used for Haemophilus influenza:**
   a. Blood agar
   b. Chocolate agar
   c. Tryptose agar
   d. BYCE agar *[Ref. Ananthanarayan, 9/e p 330]*

8. **Satellitism is seen in culture of:**
   a. Hemophilus
   b. Streptococcus
   c. Klebsiella
   d. Proteus *[Ref. Ananthanarayan, 9/e p 328]*

9. **Safety pin appearance is seen in:**
   a. Vibrio vululfieus
   b. Vibrio cholera
   c. Pseudomonas aeuroginosa
   d. H. ducreyi

Safety pin Appearance
- Yersinia pestis.
- Vibrio parahemolyticus
- Burkholderia mallei
- Klebsiella granulomatis
- Hemoptilus ducreyi

10. **Satellitism is seen in cultures of:**
    a. Hemophilus b. Streptococcus
    c. Klebsiella d. Proteus
    [Ref. Ananthanarayan, 9/e p 328]

11. **Causative organism of which STD has safety pin appearance:**
    a. LGV b. Chancroid
    c. Syphilis d. Herpes genitalis
    [Ref. Ananthanarayan, 9/e p 331]

12. **School of fish appearance is characteristic of:**
    a. Bordetella pertussis
    b. Yersinia enterocolitica
    c. Haemophillus ducreyi
    d. Legionella *[Ref. Ananthanarayan, 9/e p 331]*

13. **Brazilian purpuric fever is caused by:**
    a. Bordetella pertussis
    b. Haemophilus aegypticus
    c. Haemophilus duceryi *[Ref. Ananthanarayan, 9/e p 331]*
    d. Haemophilus parinfluenzae

Answers 1. **c.** Invasive strain... 2. **c.** Bordetella pertussis 3. **a.** B. pertussis 4. **b.** Bordettela pertussis
5. **a.** H. ducreyi 6. **c.** Invasive strain... 7. **b.** Chocolate agar 8. **a.** Hemophilus
9. **d.** H. ducreyi 10. **a.** Hemophilus 11. **b.** Chancroid 12. **c.** Haemophillus ducreyi
13. **b.** Haemophilus...

14. Granuloma inguinale is caused by:
a. H. ducreyi
b. Chlamydia trachomatis
c. Treponema pallidum
d. Calymmatobacterium *[Ref. Greenwood, 18/e p 330]*

15. Painful vaginal ulcer with inguinal lymphadenopathy and school of fish appearance of microorganism or microscopy are characteristic of:
a. Syphilis
b. LGV
c. Granuloma inguinale
d. Chancroid *[Ref. Ananthanarayan, 9/e p 331]*

16. Hemophilus influenzae requres:
a. Factor V
b. Factor X
c. Factor V & X
d. Factor VII
[Ref. Greenwood, 18/e p 325]

17. Pseudobubo seen in:
a. Chancroid
b. Syphilis
c. Lymphogranuloma inguinale
d. Lymphogranuloma venerum
[Ref. Harrison, 19/e p 881]

18. Penile ulcer is painful in:
a. Syphilis
b. LGV
c. Donovanosis
d. Chancroid *[Ref. Harrison, 19/e p 880]*

Answers **14.** **d.** Calymmatobacterium **15.** **d.** Chancroid **16.** **c.** Factor V & X **17.** **c.** Granuloma venerum **18.** **d.** Chancroid

CHAPTER

19 Campylobacter and Helicobacter

I

Campylobacter:
- Gr (–)ve, non-sporing bacilli that are motile with a single flagellum
- Show darting motility
- Grows best at 37–42°C under microaerophilic condition
- Infective dose of *C. jejuni*: 500-800 viable bacilli

CAMPYLOBACTER

- Motile, curved rods; non-sporing (comma shaped on S shaped) shows *darting motility.* with single polar flagellum.
- Important pathogenic organisms are *C. jejuni* and *C. fetus.*

CAMPYLOBACTER JEJUNI

Comma's or gull-wing shaped bacteria motile with single polar flagellum at one or both ends *...Greenwood 18/e 305*

- Very common cause of diarrhea, especially in west.
- Readily take surrounding naked DNA and gets incorporated into genome making them genetically diverse.

Culture

- Growth occurs under microaerophilic condition 5% O_2 optimal with added CO_2 [10%]. Although *C. jejuni* grows well at 36-37° C, but incubation at 42°C prevents most of other fecal bacteria thus helps in selective growth.
- ***Selective media*** – Skirrow's medium; Butzler's media; Campy BAP ***(selective media)***

Pathogenesis and clinical feature

- Infection is acquired by oral route. *Source of infection is raw or under cooked food products from poultry, cattle sheep, swine.* ***Most common being raw milk.*** *Though organism is susceptible to gastric acid* as few as **500–800** organism can produce infection when ingested with bolus of food, which protects them from acid. *...Greenwood 18/e 306*
- Organism multiply in small intestine, invade the epithelium and cause inflammation.
- Usually present: within 2-4 days.
- ***Clinically*** present as acute onset of crampy abdominal pain, profuse bloody diarrhoea.

Diagnosis

- Faeces show polymorphonuclear leucocytes.
- Gram stained smear may show ***typical 'S' shaped*** bacteria.
- Dark field or phase contrast microscopy may show *darting motility.*
- *Culture on selective media at 42°C* is **definitive diagnostic test.**
- In cases of delay, specimen should be transported in Carry Blair transport media

Treatment

- ***Erythromycin is DOC.***

CAMPYLOBACTER FETUS

- Oppurtunistic pathogen that cause systemic infection in immunocompromised.
- Cause diarrhoeal illness in normal host.
- Cause abortion in cattle

HELICOBACTER PYLORI

- Discovered by Warren & Marshall in 1982 for which they were awarded with Noble prize in 2005.

- Spiral shaped gram (–)ve microaerophilic rod associated with gastritis and peptic ulcer.
- Motile with ***lopotrichous flagella***.
- The sole source is human gastric mucosa.

Culture:
- Grows well when incubated at 37°C in microaerophilic condition.
- Media used include skirrow's medium, chocolate medium.

Biochemical reaction: Catalase (+)ve, Oxidase +ve, Urease +ve

I

Helicobacter pylori:
- Gram (–)ve, rod that is motile with lopotrichous flagella.
- Vacuolating cytotoxin is the major virulence factor.
- Associated with 80% of duodenal ulcer and 60% of gastric ulcer.

Pathogenesis

- Grows optimally at pH 6.0-7.0 and are likely to be killed at gastric pH.
- But it survives as it is found deep in mucus layer near epithelial surface, without invading mucosa where physiologic pH is present. Produces potent urease which provides ammonia to buffer acid.
- Major disease associated *H.pylori* ***virulence factor*** are vacuolating cytotoxin ***(Vac A)*** and group of genes termed CagPaI.
  - *H pylori* colonization decrease somatostatin producing cells → ↑ Gastrin → ↑ Acid → Gastric metaplasia in duodenum → Inflammation → Ulceration.
  - ***Best characterized host determinant*** of disease is enhanced *H. pylori* stimulated secretion of IL - 1 β in some people due to genetic polymorphism.

  ..... *Harrison 17/e, p 946, 18/e, p 1261*
- Colonization induce chronic superficial gastritis which includes both mononuclear and polymorphonuclear cell infiltration. ***Gastric antrum is MC site of*** colonization.

Clinical Manifestation

- ***80% of duodenal*** ulcer and ***60% of gastric*** ulcer are related to *H. pylori*.
- ***Increase risk of gastric adenocarcinoma, Gastric MALT lymphoma, autoimmune gastritis***
- Extragastrointestinal pathologies that are linked include ischemic heart disease and cerebrovascular disease, but association is not confirmed.

Diagnosis

Invasive test	***Non-invasive tests***
• Requires upper GI endoscopic biopsy	• Most consistently accurate test is urea breath test
• *Most convenient biopsy based test is biopsy urease test*	• Stool antigen test is less expensive and more convenient but slightly less accurate
• Microbiologic culture is ***most specific*** but insensitive	• Simplest tests are serologic assays measuring IgG levels in serum by ELISA or immunoblot

Urea breath test, the stool antigen test, and biopsy based tests can all be used to assess the success of treatment. However for accurate results these test should be performed only after 4 weeks of uninterrupted treatment.

Treatment

Symptomatic cases		
First line:		
Regimen 1 -	14 days	PPI BD, Clarithromycin 500 mg BD, Amoxicillin 1g BD (or Metronidazole 500 mg BD in penicillin allergic)
Regimen 2 -	10 days	PPI BD, Amoxicillin 1g BD (days 1-5), days 6-10 Clarithromycin 500 mg + Metronidazole 500 mg both BD
Second line:	14 days	Omeprazole, Bismuth, Tetracycline, Metronidazole (OBTM)

*PPI - Proton pump inhibitor

No treatment is given for asymptomatic cases.

Remember: *H. pylori* and *H. influenzae* are bacteria whose complete genome has been mapped

Multiple Choice Questions

Campylobacter

1. **A child was diagnosed to be suffering from diarrhea due to *Campylobacter jejuni*. Which of the following will be the correct environmental conditions of incubation of culture plates of the stool sample:** [AI 05]
   a. Temperature of 42°C and microaerophilic
   b. Temperature of 42°C and 10% CO_2
   c. Temperature of 37°C and microaerophilic
   d. Temperature of 37°C and 10% CO_2

2. **Darting motility occur in *V. cholerae*, also found in:** [PGI Dec 2008]
   a. *Shigella*
   b. *Campylobacter jejuni*
   c. *Pneumococcus*
   d. *Bacillus anthrax*
   e. *Aeronomas*

3. ***Campylobacter* culture media are:** [PGI Dec 2008]
   a. Schaedler's agar
   b. CVA medium
   c. Regan-Lowe medium
   d. Skirrow medium
   e. *Compylobacter* blood agar

4. **All are true statement about *Campylobacter jejuni*, except:** [AIIMS Nov 09, May 11]
   a. Human is the only reservoir
   b. Can cause GB syndrome
   c. Poultry is the source of infection
   d. Common cause of campylobacteriosis

5. **A 35-year-old patient complaints of abdominal cramps along with profuse diarrhea. Treating physician wants to process the stool specimen for isolation of *Campylobacter jejuni*. Which of the following is method of choice for culture of stool:** [AIIMS 04]
   a. Culture on TCBS media incubated at 37°C on aerobic condition
   b. Culture on Skirrow's medium incubated at 42°C under microaerophilic condition
   c. Culture on MacConkey medium incubated at 42°C under anaerobic condition
   d. Culture on Wilson and Blairs medium at 37°C under microaerophilic condition

Helicobacter

6. ***Helicobacter pylori* is not associated with:** [AIIMS 03]
   a. Gastrointestinal lymphoma
   b. Gastric cancer
   c. Gastric leiomyoma
   d. Peptic ulcer

7. **Which of following is false regarding H. pylori:** [AIIMS 00]
   a. With chronic infection urease breath test become negative
   b. *H. pylori* infection remains lifelong if untreated
   c. Endoscopy is diagnostic
   d. Toxigenic strains usually cause ulcer

8. **Which of the following correctly describe *H. pylori*:** [PGI 05]
   a. Gram-negative cocci curved
   b. Gram-positive cocci straight
   c. Gram-positive bacilli curved
   d. Gram-negative bacilli straight
   e. Gram-negative bacilli curved rod

9. ***H. pylori* true about:** [PGI 04]
   a. Gram (+)ve spiral organism
   b. It is a protozoa
   c. Causes chronic gasteritis in adults due reinfection
   d. Treatment prevents gastric Lymphoma
   e. Duodenal mucosa normal

10. **True about *H. pylori*:** [PGI 04]
    a. It is flagellated
    b. Involved in causation of peptic ulcer disease
    c. Hypergastrinemia caused by it
    d. It is a Gram (–)ve organism

11. **Regarding *H. pylori*, all are true except:** [PGI 02]
    a. Gram-negative bacillus
    b. Strongly associated with duodenal ulcer
    c. Associated with lymphoma
    d. C-14 urea breath test is used in diagnosis
    e. It should be eradicated in all cases whenever detected

12. **True about *H. pylori*:** [PGI 00]
    a. Seen in 85 to 90% cases of gastric ulcer
    b. Seen in 20 to 25% cases of duodenal ulcer
    c. Transmitted from man to man, feco-orally and by oro-gastric route
    d. Common in adults of developing countries

13. **Microaerophilic bacteria:** [AIIMS May 09]
    a. *Campylobacter*
    b. *Vibrio cholera*
    c. *Pseudomonas*
    d. *Salmonella*

14. **True statement about *H. pylori*:** [PGI 09]
    a. 75% of ulcers are a/w *H. pylori*
    b. Medical therapy is Tx of choice
    c. 90% cases of duodenal ulcer a/w *H. pylori*
    d. 60% of *H. pyulori* colonization develop ulcer

15. **Gastric ulcers of *H. pylori*, True:** [PGI 09]
    a. Gm+ve aerobes
    b. Invade mucosa and muscularis layers
    c. Rapid urease test on endoscopy is diagnostic
    d. Culture confirm eradication
    e. Causes MALT of stomach

Explanations and References with Illustrative Answers

1. Ans. (a) Temprature of 42°C and microaerophilic *Ref. Jawetz; 27/e, p 257; 25/e, p 239*

Diagnosis of *C. jejuni* diarrhoea:

- Specimen: Stool
- Direct microscopy: Dark field or phase contrast microscope shows *gull-wing shaped C. jejuni* with characterstic *darting motility*.
- Culture: Growth occurs under microaerophilic conditions 5% O_2 and 85% N_2 with added CO_2 (10%). Though *C. jejuni* grows well at 36-37°C but incubation at 42°C prevents growth of other faecal bacteria and thus helps in selective growth.

Culture Media for Campylobacter
Selective Media
1. ***CVA medium*** (*Cefoperazone – Vancomycin – amphotericin B*)
2. Skirrow medium
3. Charcoal cefoperazone deoxycholate agar (CCDA) medium
4. Charcoal based selective medium (CSM)
5. Charcoal based medium containing cefoperazone, amphotericin & teicoplan (CAT) medium
6. Campy BAP selective media
Enrichment Culture
1. Preston enrichment medium
2. Compy-thio medium
3. Campylobacter enrichment broth

- Fecal specimens are the preferred sample for isolating campylobacter species from patients with gastrointestinal infections.
- Campylobacter species primarily *C. fetus, C. jejuni* have also been isolated from blood.

Remember: *C. jejuni* cause inflammatory diarrhoea (i.e. presence of WBC in stool).

2. Ans (b) *Campylobacter jejuni* *Ref. DR Arora 3/e, p 411, 415*

Bacteria Showing Darting motility
– Spirillum minus (causative agent of rat bite fever)
– *Campylobacter jejuni*
– *Vibrio cholerae*
Mnemonic: Spicavi

3. Ans (b), (d) and (e) CVA medium, Skirro medium, Campylobacter blood agar

Already explained

4. Ans. (a) Human is the only reservoir *Ref. Harrison 19/e p 1059; Ananthnarayan 8/e p 398, 9/e p 399*

"Campylobacter jejuni is part of normal intestinal flora of domestic animals and birds including poultry, cattle, sheep and swine. Human infection is acquired through raw or under cooked meat or other food products."

- *Campylobacter jejuni* is the most common cause of *Campylobacter* infection (campylobacteriosis).
- *Campylobacter* fetus infection is common in immunocompromised patients.

Complications of Campylobacter infection

- **Generalised:** Bacteremia - (Common with C. fetus)
- **Local suppurative:** Cholecystitis; pancreatitis, cystitis

- **Distant:** Meningitis, endocarditis, arthritis, peritonitis, cellulitis, septic abortion; hepatitis; interstitial nephritis.
- **Immunologic:**
  - Reactive arthritis
  - GB syndrome (specially after C. 019 subtype)
  - Immunoproliferative small intestinal disease (α chain disease)

Infectious causes of GB syndrome	
Bacterial	**Viral**
Campylobacter jejuni	CMV
Mycoplasma pneumoniae	Epstein-Barr virus

5. **Ans. (b) Culture on Skirrow's medium incubated at 42°C under microaerophilic condition** *Ref. Jawetz 27/e, p 257; 25/e, p 23*

Already explained

Note: Skirrow's medium contains vancomycin, polymycin B and trimethoprin to inhibit growth of other bacteria.

6. **Ans. (c) Gastric leiomyoma** *Ref. Harrison 19/e, p 1039, 18/e, p 1262*

Important features of *H. pylori*:

- Gram (–)ve cocobacilli motile with lophotrichous flagella.
- 80% of *duodenal ulcer* and 60% of *gastric ulcer* are related to *H. pylori*.
- *Increase the risk of:*
  - Gastric adenocarcinoma
  - Reflux esophagitis
  - Oesophageal adenocarcinoma
  - Gatric MALT lymphoma
  - Autoimmune gastritis
- Urea breath test is most consistently accurate test for diagnosis.
- Microbiologic culture is most specific but insensitive.

7. **Ans. (a) With chronic infection urease breath test becomes negative** *Ref. Harrison 19/e, p 1040, 18/e, p 1263*

Urease breath test is most consistently accurate test for diagnosis of H. pylori.

It becomes negative only after treatment and is used to assess outcome of treatment.

Remember:
- Most specific test is microbiologic culture of specimen obtaining by upper GI endoscopic biopsy.
- Major virulence factor of *H. pylori* are vacuolating cytotoxin (VaCA) and group of genes called *CagPal*.
- *Urea breath test, stool antigen test, and biopsy based tests can all be used to assess the success of treatment.*

DIAGNOSIS OF H. PYLORI		
Test	***Advantages***	***Disadvantages***
Invasive (based on endoscopic biopsy)		
Biopsy urease test	Quick, simple	Not fully sensitive before 24h
Histology	May give additional histologic information	Sensitivity dependent on experience & use of special stains
Culture	Permits determination of antibiotic susceptibility	Sensitivity dependent on experience
Noninvasive		
Serology	Inexpensive and convenient	Cannot be used for early follow-up;
^{13}C or ^{14}C urea breath test	Inexpensive and simpler than endoscopy; useful for follow-up after treatment	Radiation exposure in ^{14}C test
Stool antigen test	Inexpensive & convenient; useful for follow-up after treatment; may be useful in children	New test; appears less accurate than urea breath test

8. **Ans. (e) Gram-negative bacilli curved rod** *Ref. Ananthnarayan 8/e, p 399, 9/e p 400*

"H.pylori is a gram-negative curved spiral rod motile by unipolar tuft of lopnotrichous flagella."

Correctly speaking *H. pylori* is a coccobacilli.

Remember:

Other Gram (–)ve coccobacilli	
– *Hemophilus*	– *Bordetella*
– *Brucella*	– *Campylobacter*
– *Helicobacter*	– *Legionella*
– *Rickettsiaceae*	– *Chlamydiae*

All important bacilli are gram negative except	
– Actinomycetes	– *Bacillus*
– *Clostridium*	– *Corynebacterium*
– Mycobacteria	– *Listeria*
Mnemonic – ABC CML	

9. Ans. (c) and (d) Causes chronic gastieritis in adults due reinfection; and Treatment prevents gastric Lymphoma

Ref. Harrison 19/e, p 1039,18/e, p 1262; CMDT 2010 551

- *H. pylori* causes type B or antrum predominant gastritis.
- MALT lymphoma (associated with *H. pylori*) remains dependent upon the presence of *H. pylori* and its eradication is often associated with complete regression of tumor.

Treatment of *H. pylori*

***Symptomatic causes* –** *Ref. Harrison 19/e, p 1042*

First line:				
Regimen 1 -	O C A	7 days	(Omeprazole, Clarithromycin, Amoxicilline)	x 7 days
Regimen 2 -	O C M	7 days	(Omeprazole, Clarithromycin, Metronidazole)	x 7 days
Second line:	OBTM	14 days	(Omeprazole, Bismuth, Tetracycline, Metronidazole)	x 14 days

Asymptomatic cases – *no treatment*

10. Ans. (a), (b), (c) and (d) It is flagellated; Involved in causation of peptic ulcer disease; Hypergastrinemia caused by it; and it is a gram (–)ve organism *Ref. Harrison 19/e, p 1039, 18/e, p 1261-1262*

***H. pylori* is associated with hyperacidity.**

H. pylori induced inflammation diminishes the number of somatostatin producing D cells. Since somatostatin inhibits the release of gastrin, patients with reduced D cells (or Helicobacter pylori +) have an increased acid secretion.

Other options have already been described.

11. Ans. (e) It should be eradicated in all cases whenever detected *Ref. Harrison 19/e, p 1041, 18/e, p 1264*

In asymptomatic cases no treatment is given.

Remember:
- The most clear cut indication of *H. pylori* treatment are *H. pylori* related duodenal or gastric ulceration or low grade gastric lymphoma.
- Family history of dyspepsia.
- *H. pylori* treatment may provoke or exacerbate GERD.

12. Ans. (c) and (d) Transmitted from man to man, feco-orally and by orogastric route; Common in adults of developing countries *Ref. Ananthnarayan 8/e, p 400, 9/e, p 406; Harrison 19/e, p 1039, 18/e, p 1261*

Mechanism of transmission is likely to be oral-oral or fecal-oral.
- 80% of **duodenal** ulcer and 60% of gastric ulcer are associated with *H. pylori*.
- Prevalance of *H. pylori* in developing countries is 80%.
- H. pylori is virtually always associated with chronic active gastritis but only 10-15% of infected individual develop frank ulceration.

13. Ans. (a) *Campylobacter*

Microaerophilic organism are organisms that require oxygen to survive, but in concentration less than that present in atmosphere, i.e. less than 21%.

Example include:

- Treponema pallidum
- Borrelia burgdorferi
- Helicobacter pylori
- Campylobacter

Mnemonic: Temp. Helicopter bording camp.

14. Ans. (a) and (b) 75% of ulcers are a/w *H. pylori*, Medical therapy is Tx of choice *Ref. Harrison 18/e, p 1263; 19/e 1039*

- *H. pylori* is associated with 80% of duodenal ulcer and 60% of gastric ulcer or it can be said that 75% (Mean of 80% and 60%) of peptic ulcers are a/w *H. pylori*.
- It is also worth to mention that only 10-15% of individual infected with *H. pylori* develop peptic ulcer.

15. Ans. (c) and (e) Rapid urease test on endoscopy is diagnostic, Causes MALT of stomach

Ref. Harrison 19/e p 1039, 18/e, p 1263

- Under normal circumstances *H. pylori* do not invade gastric mucosa. It is found deep is the mucous layer near the epithelial surface where physiologic pH is present.

Chapter Review

1. **About *Helicobacter pylori*, all are true except:**
   a. Is a Gram –ve spiral bacteria **[AIIMS 96]**
   b. Uncommon in developing Asian countries
   c. Urea breath test +ve
   d. Associated with duodenal ulcer
   [Ref. Harrison 18/e, p 1261]

2. **Which of the following has potent urease activity:**
   a. *Proteus mirabilis* [MP 06]
   b. *Brucella melitensis*
   c. *Helicobacter pylori*
   d. *Ureaplasma urealyticum*
   [Ref. Ananthnarayan 8/e, p 399, 9/e, p 400]

3. **Which of the following organism is strongly urease positive?** **[Delhi 08]**
   a. *H. pylori* b. *Proteus*
   c. *Bordetella pertusis* d. *Brucella*
   [Ref. Ananthnarayan 8/e, p 399, 9/e, p 400]

4. **All of the following are true about *Helicobacter pylori* *except*:** **[AI 98]**
   a. About 50% of world population affected
   b. 85% of population is affected, in some developing countires
   c. All children in developing countries have immunity by five years of age
   d. Infection is common in low socio-economic status
   [Ref. Harrison 18/e, p 1261]

5. **True about *Helicobacter pylori* is:** **[AI 98]**
   a. Culture and gram staining of biopsy is the gold standard investigation
   b. Controlled urea breath is negative with massive infection
   c. Anti urease antibody are produced only by invasive strains
   d. Urease activity provides protective environment to the bacilli *[Ref. Harrison 18/e, p 1261]*

6. **True about *Helicobacter pylori* are all *except*:**
   a. Urea, breath test is diagnostic **[AI 98]**
   b. Gram negative, flagellated bacilli
   c. Risk factor for development of adenocarcinoma of stomach
   d. It provides life-long immunity
   [Ref. Harrison 18/e, p 1261]

7. **The most sensitive test for *H. pylori* is:** **[PGI 99]**
   a. Breath test
   b. Rapid urease test
   c. Culture of biopsy
   d. Microscopy of biopsy
   [Ref. Harrison 18/e, p 1261]

8. ***H. pylori* causes all except:** **[DNB 09]**
   a. Gastric ulcer
   b. Duodenal ulcer
   c. Gastric cancer
   d. Colorectal cancer
   [Ref. Harrison 18/e, 1262]

9. **Which of the following is a selective media for campylobacter:** **[UP 2012]**
   a. McConkey's medium
   b. Skirrow
   c. Buffered charcoal yeast agar
   d. Choclate agar *[Jawetz. 25/e, 239]*

Answers 1. b. Uncommon ... 2. c. Helicobacter ... 3. a. H. pylori 4. c. All children.. 5. d. Urease activity...
6. d. It provides... 7. b. Rapid... 8. d. Colorectal 9. b. Skirrow

NEET Pattern Questions

1. **True about *H. pylori* includes all *except*:**
   a. Gram positive bacillus
   b. Urease positive bacillus
   c. Highly associated with deodenal ulcer
   d. Urease breath test can be performed only in specialized labs

   [Ref. Ananthanarayan, 9/e, p 316]

2. **True about Campylobacter jejuni:**
   a. Exclusively found in jejunum only
   b. Shows darting motility by peritracheal flagella
   c. Can grow at temperature around 42°C
   d. Common cause of diarrhea in India

   [Ref. Ananthanarayan, 9/e, p 399]

Darting motility is due to single polar flagellum

3. **Seven sheathed flagella is seen in:**
   a. V cholera
   b. H pylori
   c. Ps aeroginosa
   d. Spirochetes

   [Ref. Ananthanarayan, 9/e, p 400]

H. pylori has five to seven sheathed polar flagella

4. **Culture medium for Campylobacter jejuni:**
   a. BYCE medium
   b. Skirrow's medium
   c. Thayer-Martin medium
   d. TCBS medium

   [Ref. Jawetz, 27/e, p 257]

5. **True about Campylobacter jejuni:**
   a. Obligate aerobe
   b. Oxidase negative
   c. Grows at 42°C
   d. Non-motile

   [Ref. Jawetz, 27/e, p 257]

6. **True about campylobacter:**
   a. Polar flagella
   b. Grows at 25°C
   c. Strict aerobe
   d. Psychrophilic

   [Ref. Greenwood 18/e, p 305]

Campylobacter are thermophilic, as they grow best at 37–42°C

7. **Which of the following is microaerophilic:**
   a. *E. coli*
   b. Bacteroides
   c. *Clostridium*
   d. *Helicobacter pylori*

   [Ref. Greenwood 18/e, p 309]

Psychrophilic bacteria: Bacteria that grow at low temperature (< 15°C) of e.g. listeria moncytogenes

8. **Temperature required for isolation of compylobacter:**
   a. 20°C
   b. 25°C
   c. 37°C
   d. 42°C

   [Ref. Greenwood 8/e, p 305]

Answers 1. **a.** Gram positive... 2. **c.** Can grow at... 3. **b.** H pylori 4. **b.** Skirrow's medium 5. **c.** Grows at 42°C
6. **a.** Polar flagella 7. **d.** Helicobacter pylori 8. **d.** 42°C

CHAPTER

20

Legionella

- Gram-negative non-capsulated, intracellular cocobacilli.
- They are motile, aerobic, catalase and oxidase positive.

Culture

- Not grow on ordinary media.
- Buffered charcoal yeast extract *'BCYE'* is selective medium used to grow *Legionella*. It grows best at pH - 6.9, temperature 35°C and 90% humidity, 5% CO_2
- Colonies have a 'cut glass' appearance and exhibit blue white autofluorescence

...Greenwood 18/e, p 340

- ***MC*** species associated with human infection *is L. pneumophilia,* (***MC*** with ***serogroup 1***).
- Other important species is *L. micdadei* (Pittsburgh pneumonia agent). It is partial acid fast (AFB).
- ***Natural habitat*** is *aquatic bodies including lakes and streams.* Shows symbiotic relations with algae, amebas, ciliated protozoa.
- Factors enhancing colonization are warm temperature, stagnation and sediments. It can form *microcolonies within biofilms.* Its eradication require disinfectants that can penetrate the biofilm.

I

- Gram (–)ve, non motile aerobic intracellular coccobacilli
- Buffered charcoal yeast agar is selective medium
- BCYE contains iron and cysteine

Mode of Transmission

- ***Aspiration*** is predominant mode of transmission. Other modes include aerosolization, direct instillation into lungs.
- *No man-to-man transmission occurs.*
- Aerosolization by AC, nebulizer, humidifier, overhead showers and direct installation into lung are other modes.

I

- *L. pneumophila* is the most common *Legionella* species associated with human infection
- Aspiration is predominant mode of transmission.

Risk Factors and Pathogenesis

- Conditions that impair mucociliary clearance predispose to legionnaires disease, most commonly being cigarette smoking.
- ***Hairy cell leukemia*** (not other leukemia) and immunocompromised state are other risk-factors.
- Legionella enters the lung through aspiration or direct inhalation. Attachment to host cells is mediated by bacterial type IV pilli, heat shock proteins, and the major outer membrane protein. *Legionella* then binds complement CR1 and CR3 integrin receptors of phagocytic cells. Entry into the cell is by phagocytic process. Alveolar macrophages readily phagocytose *Legionella* but bacteria actively multiply with in macrophages, when cells are destroyed the bacteria are released and infect other macrophages.
- The presence of iron is essential for intracellular growth of the bacteria.
- *Cell mediated immunity is primary mechanism of host defense* (Role of neutrophil appears to be minimal).
- Humoral immunity plays no role.

I

- *L. pneumophila* is oxidase positive (others are variable)
- *L. pneumophila* hydrolyzes hippurate where as others do not.

Legionellosis

Legionella causes 2 clinical syndromes:

*A. **Pontiac fever:***

- Acute febrile self-limited illness. Airbone transmission with high attack rate.
- Pneumonia does not develop. ***MC* symptom** – malaise, fatigue and myalgia.

I

Legionella cause atypical pneumonia and legionnaires disease

- Heart is the most common site for extrapulmonary legionellosis
- Diarrhea with pneumonia suggest *Legionella* pneumonia.

B. ***Legionnaires' disease:*** Designation for pneumonia.

- ***4th MC*** cause of community acquired pneumonia (***MC*** – *S.pneumoniae* **2nd** ***MC*** – *H. influenzae* **3rd** ***MC*** *Chlamydia pneumoniae*). ..... *Harrison 17/e, p 929*
- Cause atypical pneumonia which is more serious than atypical pneumonia of other agents.
- ***Clinical features suggestive of*** **L. pneumonia:**
  - Diarrhea – High fever
  - Hyponatremia – Proteinuria
  - Onset of symptom if occur within 10 days after discharge from hospital, suggest nosocomial legionnaires disease.
- Mostly caused by serotype 1 but serotype 6 is associated more commonly with hospital acquired and has poor prognosis.
- Relative bradycardia is useful diagnostic finding.

Extrapulmonary Legionellosis

- Results from blood-borne dissemination from lung.
- ***MC*** extrapulmonary site is **heart (myocarditis, pericarditis).**
- Most cases are hospital acquired.
- ***MC*** neurological **abnormality** are **confusion** or changed mental status.

Diagnosis

Specimen	Sensitivity of bronchoscopy specimens is approximately the same as that of sputum. Bronchoalveolar lavage fluid gives higher yield than bronchial wash specimen.
Staining	Gram-staining usually show numerous leukocytes but no organisms.
	DFA test is rapid and highly specific but less sensitive.
Culture	*Definitive* method of diagnosis.
	Requires 3-5 days to become grossly visible.
	B'CYE media is used.
Antibody detection	Requires 12 weeks, so used for retrospective diagnosis or epidemiologic studies.
Urinary antigen	Cheap, rapid, second in sensitivity and highly specific.
	Detectable within 3 days.
	The test is not affected by antibiotic administration.
Radiographic abnormalities	Pleural effusion
	In immunosuppressed distinct round nodular opacity may be seen.

Treatment

- Azithromycin and respiratory tract quinolones (Levofloxacin, gemifloxacin, moxifloxacin) are *DOC.*
- Quinolones are preferred antibiotic in transplant recipients.

Prevention

- Disinfection of water by:
  - Superheat and flush method – ***Ideal*** for ***emergency*** situation
  - Copper and silver ionization method
  - Tap water filters particularly in transplant units.

Note: ***Superchlorination*** is ***not*** effective against ligionella.

Multiple Choice Questions

1. **A 70 years old patient presents with high grade fever, dry cough and abdominal pain. Sputum sample collected from patient, shows gram negative organisms that are able to grow only on charcoal yeast extract medium. The most likely organism is:** [AI 07; AIIMS 06]
   a. *H. influenza*
   b. *Legionella*
   c. *Lesteria monocytogenes*
   d. *M. catarrhalis*

2. **A 60-year old man is diagnosed to be suffering from Legionnaires disease after he returns home from attending a convention. He could have acquired it:** [AI 03]
   a. From a person suffering from the infection while traveling in the aeroplane
   b. From a chronic carrier in the convention center
   c. From inhalation of the aerosol in the air conditioned room at convention center
   d. By sharing an infected towel with a fellow delegate at the convention

3. **Anju, a 28 years female, has diarrhea, confusion, high grade fever with bilateral pneumonitis. Organism causing this:** [AI 00]
   a. *Legionella*
   b. *Neisseria meningitidis*
   c. *Streptococcus pneumoniae*
   d. *H. influenzae*

4. **All of the following are true regarding *Legionella* except:** [AIIMS 04]
   a. *Legionella* can be grown on complex media
   b. *L. pneumophila* serogroup 1 is the most common serogroup isolated from humans
   c. *Legionella* are communicable from infected patient to others
   d. *L. pneumophila* is not effectively killed by polymorphonuclear leukocyte

5. **Which of the following is a good media to use for diagnosis of legionnaires disease:** [AIIMS 01]
   a. Thayer Martin media
   b. BCYE agar
   c. Bordet Gengou media
   d. Chocolate agar

6. **Pontiac fever is caused by:** [PGI Dec. 07]
   a. *Legionella* b. *Listeria*
   c. *Scrub typhus* d. *Leptospira*
   e. *Rickettsia*

7. **BCYE. medium is used to culture:** [PGI 99]
   a. *Mycoplasma* b. *T pallidum*
   c. *H. pylori* d. *Legionella*

8. **Pontiac fever is caused by:** [PGI Nov 11]
   a. Listeria Monocytogenes
   b. *S. aureus*
   c. *Cl. tetani*
   d. *Legionella pneumophila*
   e. *Borrelia recurrentis*

9. **An elderly patient presented with fever, chest pain and dry cough. Sputum cultured on charcoal yeast medium, the most likely organism is:** [AIIMS Nov 11]
   a. *H. influenza* b. *Moraxella catarrhalis*
   c. *Legionella* d. Burkhoderia cepacia

10. **Aerosol spread leading to epidemics is seen in infection with:** [AIIMS Nov 2012]
    a. Legionella b. Hemophilus
    c. Influenza virus d. Mycoplasma

11. **Method of transmission of legionella includes all, except:** [AIIMS May 2013]
    a. Patients suffering from legionella to contacts
    b. Aerosol from air cooling systems
    c. From contaminated food
    d. Through contaminated tracheal tubes

Explanations and References with Illustrative Answers

1. **Ans. (b)** ***Legionella*** *Ref. Harrison 19/e, p 1014, 18/e, p 1236; Jawetz 27/e, p 301*

Dry cough, high grade fever and growth on charcoal yeast medium suggest legionella.
Legionella have fastidious requirements and grow on complex media such as buffered charcoal, yeast extract (BCYE) agar with L. cysteine and antibiotic supplements with 5% CO_2 at pH 6.9, 35°C and 90% humidity.

2. **Ans. (c) From inhalation of the aerosol in the air conditioned room at convention center** *Ref. Harrison 19/e, p 1015, 18/e, p 1237*

Important features of *Legionella*
- Aerobic, Gram-negative motile, nonencapsulated bacilli
- Natural habitat are aquatic bodies such as stagnant water, mud, hot springs.
- Outbreaks are associated with contaminated water source such as air conditioning cooling towers.
- Multiple modes of transmission—***Aspiration (MC)***, aerosolization, direct instillation.
- ***No man-to-man transmission,*** no animal reservoir.
- It causes:

Manifestations	
Pneumonia	***Pontiac fever***
Atypical pneumonia	Acute febrile self-limiting illness
Presents with high fever, diarrhea, pneumonia	
MC extrapulmonary site of Legionella is heart.	

- **Selective media** – Buffered charcoal yeast extract **(BCYE) agar.**

Treatment: – Macrolides and quinolones.
– β-lactams are not effective.

3. **Ans. (a)** ***Legionella*** *Ref. Harrison 19/e, p 1016, 18/e, p 1237*

Clinical features suggestive of Legionnaire's disease
- Diarrhea
- High fever (>40°C or >104°F).
- Numerous neutrophils but no organisms revealed by Gram's staining of respiratory secretions.
- Hyponatremia (serum sodium level of < 131 meq/L).
- Failure to respond to β-lactam drugs (penicillins or cephalosporins) and aminoglycoside antibiotics.
- Occurrence of illness in an environment in which the potable water supply is known to be contaminated with Legionella.
- Onset of symptoms within 10 days after discharge from the hospital.

Remember: MC extrapulmonary site in heart in which it causes myocarditis, pericarditis.

4. **Ans. (c)** ***Legionella*** **are communicable from infected patient to others** *Ref. Harrison 19/e, p 1015, 18/e, p 1236*

"Their is no man-to-man transmission."
- **Modes of transmission** are aerosolization, aspiration and direct instillation of contaminated water into the lung.
- ***L. pneumophila*** is *most common Legionella* causing human disease.
- Serogroup 1, 4 and 6 are *most commonly* implicated.
- **Cell mediated** immunity is primary mechanism of host defence. Alveolar macrophage readily phagocytose *Legionella;* many are killed but some proliferate intracellularly until cell rupture.

Risk factors for Legionnaires disease
- **Cigarette** *smoking and other condition that impair mucociliary clearance*

- Chronic lung disease
- Advances age
- Immunosuppression
- Surgery is predisposing factor in nosocomial infection with transplant recipient at highest risk.

5. **Ans. (b) BCYE agar** *Ref. Jawetz 27/e, p 301*
   - **BCYE agar** is the medium used to grow *Legionella.*
   - This highly enhanced medium contains the ***amino acid L-cysteine*** which is an ***absolute growth requirement*** for *Legionella.*
   - Addition of vancomycin, polymyxin B and an antifungal agent increases the selectivity.
6. **Ans. (a) *Legionella*** *Ref. Harrison 19/e, p 1016, 18/e, p 1237*

   Pontiac fever is a mild nonfatal influenza like illness caused by Legionella pneumophila.

   Pontiac fever
   - An acute self limiting flue like illness with IP of 24-48 hours
   - Malaise, fatigue and myalgia are the most frequent presenting symptoms
   - Pneumonia doesn't develop.
   - Complete recovery takes place, without antibiotic therapy.
   - Diagnosis is established by antibody detection.
7. **Ans. (d) *Legionella*** *Ref. Jawetz 19/e, p 301*

   Already explained
8. **Ans. (d) *Legionella pneumophilia*** *Ref. Harrison 19/e, p 1015, 18/e, p 1237*

   Already explained
9. **Ans. (c) *i.e. Legionella*** *Ref. Harrison 18/e, 1236-1237*

   Already explained
10. **Ans. (a) *Legionella*** *Ref. Harrison 18/e 1236*
    - *Aerosolization of Legionella by devices filled with tap water including whirlpools, nebulizers and humidifiers has been implicated"*
    - *Pontaic fever has been linked to Legionella containing aerosols from water using machinery, a cooling tower, AC and whirlpools.*

    Other Options:
    - **Hemophilus:** Transmitted by airborne droplets or by direct contact with secretions or fomites.
    - **Influenzae virus:** Same as hemophilus
    - **Mycoplasma:** Person to person by respiratory droplets expectorated during coughing.
11. **Ans. (a) *i.e. Patients suffering to*** *Ref. Ananthanarayan 9/e, p 401; Harrison 18/e, 1236, 19/e, p 1015*

    Human to human transmission does not occur in legionella

Mode of Infection of Legionella:

- **Source:**
  - Natural habitat for L. pneumophila are aquatic bodies including lakes and streams. However their number is very low in aquatic bodies
  - When the contaminated water from these aquatic bodies is stored in human constructed water reservoirs (water cooler), legionella grow and proliferate.
  - Warm temperature and sediment enhances the proliferation
  - L. pneumophila can form microcolonies within biofilms in water coolers, its eradication requires disinfectants that can penetrate the biofilm
  - Ameboe, alga, ciliated protozoans are symbiotic to L. pneumophila and promotes the growth
- **Mode of transmission:**
  - **Aspiration:** Predominant mode. Aspiration can occur either from oropharyngeal colonization, or through contaminated water
  - **Aerosolization:** Air conditioners, whirlpools, nebulizers aerosolize the legionella which then gets inhaled.
  - **Direct instillation into the lungs:** Either through contaminated instruments (endotracheal tube) or through respiratory tract manipulation which mobilizes the oropharyngeal colonies to respiratory tract.

NEET Pattern Questions

1. **Buffered charcoal yeast agar is the selective medium for:**
   a. Listeria monocytogenes
   b. Legionella pneumophila
   c. Pseudomonas aeroginosa
   d. T. pallidum

   [Ref. Ananthanarayan, 9/e, p 401]

2. **Which of the following pneumonia is caused by contaminated air conditioner:**
   a. Pneumococci
   b. Staphylococci
   c. E. coli
   d. Legionella

   [Ref. Harrison, 18/e, p 1236]

3. **Legionnaire disease is caused by:**
   a. Motile gram positive
   b. Motile gram negative
   c. Non-motile gram positive
   d. Non-motile gram negative

   [Ref. Ananthanarayan, 9/e, p 401]

4. **In pontiac fever, which antigen is seen in urine:**
   a. Group specific antigen of Legionella serogroup 1 (LP 1)
   b. Group specific antigen of Legionella serogroup 2 (LP 2)
   c. Group specific antigen of Legionella serogroup 4 (LP 4)
   d. Group specific antigen of Legionella serogroup 6 (LP 6)

   [Ref. Harrison, 19/e, p 1017]

5. **True about legionella:**
   a. Most common mode of transmission is aerosol inhalation
   b. There is no man to man transmission
   c. Prolonged carrier are common
   d. All are true

   [Ref. Harrison, 19/e, p 1015]

6. **Legionella causes:**
   a. Pontiac fever
   b. Myocarditis
   c. Diarrhea
   d. All of the above

   [Ref. Harrison, 19/e, p 1016]

Answers 1. **b.** Legionella... 2. **d.** Legionella 3. **b.** Motile gram... 4. **a.** Group specific... 5. **b.** There is no man... 6. **d.** All of the above

CHAPTER

21

Rickettsiae and Chlamydiae

CHLAMYDIAE [PLT AGENT]

- **Obligate intracellular** bacteria, so *unable* to *grow in cell free media.*
- Lacks enzymes of electron transport chain and require ATP from host cell, so they are often called as ***energy parasites.***
- Cell wall resemble that of Gr(-)ve bacteria
- ***Peptidoglycan and N-acetylmuramic acid is absent*** from its cell wall. Its cell wall contain tetrapeptide linked matrix and relatively high lipid content.
- Show tropism for squamous epithelial cells and lymph nodes.
- Genome of chlamydiae is 1.04 megabases in length encodes 900 genes, and is one of the smallest bacterial genomes.

I

Chlamydia
- Obligate intracellular bacilli, also called as energy parasites
- Cell wall lacks peptidoglycan and N-acetylmuramic acid

Growth Cycle

Replicate by ***binary fission*** without an eclipse phase.
Chlamydiae occur in 2 forms:

Elementary Body (EB)	Reticular Body (RB)
Extracellular metabolically inactive infective form	Intracellular growing, metabolically active and replicative form
Contain rigid trilaminar cell wall	Friable cell wall lacking peptidoglycan
Contain electron dense nucleoid	No electron dense nucleoid
DNA = RNA	RNA > DNA about 4 times

I

Chlamydia
- Elementary body usually attach near the base of microvilli

Reticular body undergoes binary fission resulting in chlamydial microcolony called ***inclusion body.*** This whole cycle takes about 24 - 48 hours.

Effect on Host Cell

- *C. trachoma* leave host cell with scar while *C. psittacosis* leaves host cell severely damaged which is usually followed by lysis.

Classification

Chlamydiae are divided into four species:

i. *C. trachomatis* ┐
ii. *C. pneumoniae* ├ (Affect humans)
iii. *C. psittaci.* ┘
iv. *C. pecourum* (Affect ruminants)

Features	C. trachoma	C. pneumoniae	C. psittaci
Serovars	15	1	≥ 4
Inclusion body	Round vacuolar called *HP bodies	Round dense	Large dense called *LCL bodies
Glycogen in inclusions	+	–	–
Susceptibility to sulfonamide	+	–	–
Plasmid	+	–	+
Natural host	Humans	Humans	Birds
Transmission	Person-to-person Mother to infant	Airborne person-to person	Airborne bird excreta to humans
Elementary body morphology	Round	Pear-shaped, round	Round

*HP = Halberstaedter Prowazek
*LCL = Levinthal - cole - lillie

I

Chlamydia
- Elementary body: Infective entity
- Reticular body: Metabolically active entity.

I
- C. pneumoniae grows well on HL and Hep-2 cells.
- C. trachomatis grows well on Mc Coy cells.

Antigen

- **Heat stable LPS** (Lipopolysaccharide)
  - Genus (Group) specific
  - Common to all chlamydia
  - Responsible for complement fixation test (CFT).
  - Species specific protein antigen, present at envelope surface, so classify chlamydiae into trachomatis, psittaci, pneumoniae and pecorum.
- **Major outer membrane protein (MOMP)**
  - Used for intraspecies typing, i.e. for serovar or serotypes.
  - Demonstrated by microimmuno-fluorescence.

Lab Diagnosis

- *Microscopy*
  a. Staining
  - By Giemsa or castaneda or Machiavello particularly in neonatal inclusion conjuctivitis. As C. trachomatis inclusions contain glycogen matrix they can be stained with Lugol's iodine
  - ***Iodine staining*** of conjunctival scrapping is a *rapid and simple screening method for trachoma*

  b. Immunofluoroscence
  - Using monoclonal antibody
  - More sensitive and specific.
- ***Isolation*** – *Cell culture* is the preferred mode.
  - Can also be done by inoculation into embryonated eggs or experimental animals.
  - McCoy & Hela All lines are commonly used ...*Ananthanarayan 9/e, p 418*
- ***Demonstration of antigen***
  a. Microimmunoflueroscence – Commonly used method.
  b. ELISA – Preferred for screening.
  c. DNA probes and amplification techniques (PCR and LCR) – More sensitive and specific.
- ***Detection of antibody*** – CFT: Cannot distinguish species as it is Group = Genus specific.
  - Micro IF: More useful for TRIC (inclusion conjunctivitis).

Remember:
- High titre antibody are seen only in:
  - Infant pneumonia – Salpingitis – LGV.
- *C. pneumoniae*, grows better in HL and HEp - 2 cells than in HeLa 22q or McCoy cells.
- McCoy cells are widely used to culture C. trachomatis.
- Skin hypersensitivity in LGV can be demonstrated by Frie's test.

CHLAMYDIA TRACHOMATIS

- ***MC*** cause of STD worldwide.
- ***MC*** cause of ophthalmia neonatorum.
- 15 serotypes [A to K and L1, L2, L3] are known:
  - A, B, Ba, C - Endemic blinding trachoma.
  - D to K - Inclusion conjunctivitis, genital infection, infant pneumonia.
  - L_1, L_2, L_3 Lymphogranuloma venereum.
- *C. trachomatis* is ***MC*** cause of non-gonococcal urethritis, post-gonococcal urethritis.
- ***MC*** cause of epididymitis.
- **Inclusion conjunctivitis of *neonate*** is called ***inclusion blenorrhea*** while *adult* form is called as ***"Swimming pool disease."***

I
- C. trachomatis is the most common STD worldwide, and is the most common cause of non-gonococcal urethritis
  - Associated with ophthalmia neonatorum, lymphogranuloma venereum

Clinical Manifestations

IP: 5–10 days.

1. Trachoma

- Chronic keratoconjunctivitis characterized by follicular hypertrophy, papillary hyperplasia, pannus formation and in late stage, cicatrisation

- **Stages include**
  - Trachoma dubium (earliest), when disease is just suspicion
  - Protrachoma (stage of conjunctival lesion).
  - Established trachoma (Stages I-IV)
  - Inclusion bodies are not demonstrable in trachoma dubium and protrachoma

I
- L_1–L_3 serovar are more invasive and replicates in macrophages
- Trachoma serovars are more common and replicates in eye (A–C) on genital tract (D–K)

2. Genital Infection

- Chlamydia serovars D-K causes two types of genital infection viz. LGV and miscellaneous urogenital syndrome, Collectivity referred as genital chlamydiasis.

a. **LGV - Lymphogranuloma venereum**
   - Most cases occur due to **L2** serovar–LGV serovars are more invasive than other serotypes.
   - ***MC* presentation** in heterosexual man is painful lymphadenopathy called inguinal syndrome/Tropical bubo.
   - LGV strains are **more invasive** than the other serovars.
   - ***MC*** LN involved in woman - *Intrapelvic and pararectal.*
   - In women it causes rectal stricture and elephantiasis of vulva (esthiomene).
   - Elementary bodies are known as *Miyagawa's corpuscles.*

Lymphogranuloma Venereum		
Primary stage	*Secondary stage*	*Tertiary stage*
• 3 – 30 days of IP	• 2 – 6 weeks after primary lesion	• After several years
• Painless small papule which may ulcerate at the site of lesion	• Characterized by inguinal lymphadenopathy which is painful	• Elephantiasis of genitalia
	• Proctitis	• Esthiomene syndrome
	• Cervical adenopathy after oral intercourse	

I
LCR and PCR are most sensitive chlamydial diagnostic test available

Note: Sign of groove: Extensive enlargement of chain of inguinal nodes above and below the inguinal ligament (the sign of the groove). Present only in minority of cases of LGV.

b. **Genital chlamydiais:**
   - MC form of STD worldwide
   - In ***men*** they cause urethritis (nongonococcal urethritis), epididymitis, prostitis, conjunctivitis and urethritis.
   - In ***women*** bartholinitis, mucopurulent cerviticis, endometritis, salpangitis are seen.
   - Reiter syndrome and Fitz-Hugh -curtis syndrome can be seen in both sexes.

3. Reiter's syndrome

- Conjunctivitis, urethritis, arthritis and characteristic mucocutaneous lesions
- Associated with HLA B - 27.

I
C. neumoniae: Atypical pneumoniae.
C. psittaci: Psitticosis.

Diagnosis:

- ***Cell culture once considered to be the gold standard has been replaced by NAAT.***
- ***LCR and PCR are most sensitive*** chlamydial diagnostic test available.

..... Harrison 19/e, p 1071, 18/e, p 1426

Treatment:

- Azithromycin is the *DOC* for STD.
- Tetracycline, erythromycin, rifampicin are effective in trachoma

CHLAMYDIA PNEUMONIAE (TWAR STRAIN)

- Distinguished from other 2 species on the basis of DNA morphology.
- Only one serovar identified.
- Associated with increased risk of atherosclerosis, asthma, sarcoidosis.
- **HL and HEP-2 cells** - Most effective cell line for isolation.
- Causes atypical pneumonia, similar to M pneumoniae, 5-20% of community acquired pneumonia are thought to be caused by C. pneumoniae
- Pharyngitis, otitis, sinusitis, bronchitis are other manifestation

Treatment: Erythromycin/Tetracycline.

I

Rickettsiae

- Gr (–)ve obligate intracellular parasite.
- Only louse borne typhus is the primary human disease, in other rickettsial disease, human are incidental host.
- Rocky mountain spotted fever is the most severe rickettsial disease.
- Rickettsia pox is the mildest rickettsial disease.

CHLAMYDIA PSITTACI

- Primarily disease of parrots.
- Acquired in humans by inhalation of dropping or nasal discharge.
- Human infection mostly occupational.
- Consumption of poultry products does not lead to infection.
- Psittacosis is a septicemia, pneumonia is usual manifestation.

Treatment:

- Tetracycline is *DOC*
- Alternative erythromycin.

RICKETTSIACEAE

- This family consist of 3 genera - Rickettsia, Orientia and Ehrlichia.
- These are ***Gram –ve obligate intracellular parasite*** so unable to grow in cell free media, except Rochalimaea quintana.
- Transmitted by arthropod vectors.
- In humans they infect vascular and reticuloendothelial cells.
- ***Except for louse-borne typhus, humans are incidental hosts.***
- *Coxiella burnetii* is notorious for its ability to survive outside reservoir or vector and for its extreme infectiousnes. ***(Non arthropod air-borne rickettsial disease).***
- Severity of rickettsial disease are enhanced by sulphonamide. Penicillin is also ineffective in rickettsial disease.
- Rickettsia are stained by Giemsa, Castaneda, Machiavello and Gimenez stains.
- Rickettsia grow best in cells that are not metabolizing actively.
- Rickettsia are non-motile non-capsulated, pleomorphic coccobacilli.

Classification of Rickettsial Cell Disease

I

Rickettsial disease with Eschar

- Scrub typhus.
- Indian tick typhus.
- Siberian tick typhus.
- Rickettsial pox.

	Diseases	Agents	Vectors
Typhus group	a. Epidemic typhus or Brill zinsser disease	R. prowazekii	Louse
	b. Murine/Endemic typhus	R. typhi (R. mooseri)	Flea
Spotted fever group	a. Rocky Mountain spotted	R. rickettsii Fever	Tick
	b. R. pox	R. akari	Mite
	c. Fever boutonneuse or Mediterranean spotted fever or Indian tick typhus	R. conorii	Tick
Other	a. Q. fever	Coxiella burnetii	Nil [Air Borne]
	b. Trench fever/ Five day fever	Rochalimaea quintana (Bartonella quintana)	Louse
	c. Scrub typhus (Chigger-borne typhus)	R. tsutsugamushi or Orientia tsutsuga mushi	Mite
	d. Ehrlichiosis e. Cat Scratch disease	Ehrlichiae Bartonella henslae	Tick

Note: B. henslae has been linked with bacillary angiomatosis and bacillary peliosis.

I

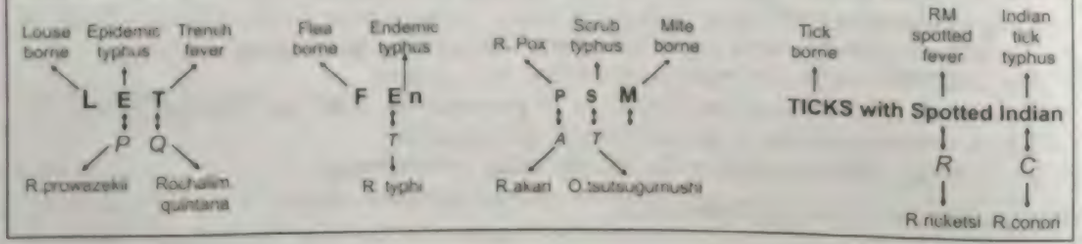
- Neil-Mooser reaction.

Tunia reaction.

- Used to differentiate R typhi and R prowazekii (–ve reaction)

- ***Don't go into DETAILS of Individual disease as they are asked very rarely.***

Mnemonic to Learn this Confusing Table



Important Points About Rickettsial Diseases

- **Most severe** rickettsial disease is Rocky mountain spotted fever.
- **Most mild** rickettsial disease Rickettsia pox.
- Rickettsial infections are characterized by fever, headache, malaise, prostration, skin, rash (except in Q fever) and hepatosplenomegaly.
- Neil - Mooser or tunica reaction positive in *R. typhi* (*R.mooseri*), *R. conori*, *R.akari* and negative for *R. prowazekii*.
- **Weil Felix reaction** - It is heterophile agglutination test. Based on sharing of alkali stable polysaccharide between typhus rickettsia and some strain of *Proteus* bacilli. (OX 19, OX - 2, OX K)
  - **OX 19:** (+++): In epidemic and endemic typhus, Tick born spotted fever.
  - **OX - 2:** (++): In rocky mountain spotted fever.
  - **OXK**: (+++): In scrub typhus.
  - *No value in:* Q fever, trench fever, rickettsial pox.
  - *False positive in:* Typhoid, liver disease, *Proteus* infection, *Pseudomonas*, *Borrelia*, *S.typhi.*

Multiple Choice Questions

Rickettsiae

1. **Lice are not the vectors of:** [AI 07; AIMS 06]
   a. Relapsing fever 4 b. Q fever
   c. Trench fever d. Epidemic typhus

2. **All are true about scrub typhus, except:** [AI 2010]
   a. Causative organism is R.TSUTSUGAMOSHI
   b. Vector is trombiculide mite
   c. Adult female feeds on vertebrate hosts
   d. Tetracycline is the drug of choice

3. **A patient complained of chills and fever following a louse bite 2 weeks before. He had rashes all over the body and was delirious at the time of presen-tation to the hospital and subsequently went into coma. A provisional diagnosis of vasculitis due to rickettsial infection was made. Which one of the following can be the causative agent?** [AI 05]
   a. *Rickettsia typhi* b. *Rickettsia rickettsiae*
   c. *Rickettsia prowazekii* d. *Rickettsia akari*

4. **All of the following statement are true regarding Q fever except:** [AI 03]
   a. It is a zoonotic disease
   b. Human disease is characterized by an interstitial pneumonia
   c. No rash is seen
   d. Weil Felix reactions is very useful for diagnosis

5. **Which of the following statements is true about endemic typhus?** [AI 03]
   a. Is caused by *R. rickettsii*
   b. Is transmitted by bites of fleas
   c. Has no mammalian reservoir
   d. Can be cultured in chemical defined culture medium

6. **True about scrub typhus:** [PGI Nov 11]
   a. Zoonotic disease
   b. Positive Well-Felix reaction
   c. Spread by adult mite
   d. Eschar indicates the location of mite bite
   e. Spread by infected chigger

7. **Scrub typhus is transmitted by:** [AIIMS Nov. 07]
   a. Reduvid bug b. Trombiculid mite
   c. Enteric pathogens d. Cyclops

8. **It is true regarding endemic typhus that:** [AIIMS 06]
   a. Man is the only reservoir of infection
   b. Flea is a vector of the disease
   c. The rash developing into eschar is a characteristic presentation
   d. Culture of the etiological agent in tissue culture is a diagnostic

9. **Following is the etiological agent of Rocky Mountain spotted fever:** [AIIMS 05]
   a. *R. rickettsii* b. *Rochalimae quintana*
   c. *R. tsutsugamushi* d. *Coxiella burnetii*

10. **Mode of transmission of Q-fever is:** [AIIMS 04]
    a. Bite of infected louse
    b. Bite of infected tick
    c. Inhalation of aerosol
    d. Bite of infected mice

11. **A man presents with fever, chills 2 weeks after a louse bite. There was maculopapular rash on the trunk which spread peripherally. The cause of this infection can be:** [AIIMS 03]
    a. Scrub typhus b. Endemic typhus
    c. Rickettsiae pox d. Epidemic typhus

12. **Disease caused by both Rickettisia and Orientia is transmitted by:** [PGI 2011]
    a. Rat flea b. Tick
    c. Louse d. Trombiculid mite
    e. Gamaxid mite

13. **Which is caused by *Rickettsia?*** [PGI 07]
    a. Weil's disease
    b. Rocky mountain spotted fever
    c. Scrub typhus
    d. Lymes disease

14. **Tick is vector for:** [PGI 2012]
    a. Crimean congo fever
    b. Rocky mountain spotted fever
    c. Epidemic typhis
    d. Endemic typhis
    e. Scrub typhus

Chlamydiae

15. **Regarding *Chlamydia* infection of the eyes, true statement include the following except:** [AI 09]
    a. Mostly asymptomatic
    b. Can be cultured
    c. Inclusion conjunctivitis is an acute ocular infection caused by sexually transmitted *C. trachomatis* strains (usually serovars D through K)
    d. Penicillin is the treatment

16. **In a patient with UTI; on smear, no bacteria are found on gram stain with abundant pus cells, to demonstrate organism, which of the following is useful?** [AI 07; AIIMS May 2012, 11, 06]
    a. McCoy cell line b. Thayer martin medium
    c. L. J. medium d. Acid fast staining

17. **Which of the following is not true regarding *Chlamydia*?**
    a. Has biphasic life **[AI 07; AIIMS 06]**
    b. Elementary body is metabolically active
    c. Reticulate body undergoes binary fission
    d. Once it invades into cell it abates phagolysosomal fusion

18. ***Chlamydia trachomatis* is associated with the following except: [AI 05]**
    a. Endemic trachoma
    b. Inclusion conjunctivitis
    c. LGV
    d. Community acquired pneumonia

19. **Which one of the following statements is true regarding *Chlamydia pneumoniae*? [AI 05]**
    a. Fifteen serovars have been identified as human pathogen
    b. Mode of transmission is by the air-borne bird excreta
    c. The cytoplasmic inclusions presents in the sputum specimen are rich in glycogen
    d. The group specific antigen is responsible for the production of complement fixing antibodies

20. **The most sensitive method for detecting cervical *Chlamydia trachomatis* infection is: [AI 04]**
    a. Direct fluorescent antibody test
    b. Enzyme immunoassay
    c. Polymerase chain reaction
    d. Culture on irradiated McConkey cells

21. **Chlamydia does not cause: [AI 00]**
    a. Q. fever
    b. Non-gonococcal urethritis
    c. Trachoma
    d. Salpingitis

22. **All are true regarding *Chlamydia psittaci* except:**
    a. Endemic in birds [AIMS 06]
    b. Cause non-gonococcal urethritis
    c. Grow in a specified laboratory
    d. Tetracycline is treatment of choice

23. **A 45 years female complains of lower abdominal pain and vaginal discharge. On examination there is cervicitis along with a mucopurulent cervical discharge. The gram smear of the discharge shows presence of abundant pus cells but no bacteria. The best approach to isolate the possible causative agent would be: [AIIMS 05]**
    a. Culture on chocolate agar supplemented with Haemin
    b. Culture on McCoy cells
    c. Culture on a bilayer human blood agar
    d. Culture on vero cell lines

24. **The following is not a method of isolation of *Chlamydia* from clinical specimens: [AIIMS 05]**
    a. Yolk inoculation
    b. Enzyme immunoassay
    c. Tissue culture using irradiated McCoy
    d. Tissue culture using irradiated BHK cells

25. **The following statements are true regarding *Chlamydia* except: [AIIMS 05]**
    a. Erythromycin is effective for therapy of Chlamydial infections
    b. Their cell wall lacks a peptidoglycan layer
    c. They can grow in cell free culture media
    d. The are obligate intracellular bacteria

26. **A man presents to STD clinic with urethritis and urethral discharge. Gram stain shows numerous pus cells but no microorganism. Culture is negative on routine laboratory media. The most likely agent is: [AIIMS 02]**
    a. *Chlamydia trachomatis*
    b. *H. ducreyi*
    c. *T. pallidum*
    d. *N. Gonorrhoeae*

27. ***Chlamydia trachomatis* serovars D-K cause:**
    a. Arteriosclerosis [AIIMS 02]
    b. *Trachoma*
    c. *Lymphogranuloma venereum*
    d. Urethritis

28. **Triad of Reiter's syndrome: [PGI 07]**
    a. Conjunctivitis
    b. Uveitis
    c. Polyarthritis
    d. Mucosal lesions
    e. Glaucoma

29. ***Chlamydia* grows in which of the following cell lines?**
    a. HeLa
    b. HeP2 [PGI 01]
    c. McCoy
    d. Human diploid fibroblast
    e. Vero cells series

30. **Chlamydia causes: [PGI 00]**
    a. Infertility
    b. Pneumothorax
    c. Pelvic inflammatory disease
    d. Congenital malformation in fetus

31. **Most sensitive test for diagnosing asymptomatic chlamydia infection is: [AIIMS 09]**
    a. Tissue culture
    b. Nucleic acid amplification
    c. Serology
    d. Serum electrophoresis

32. **Isolation of Chlamydia from tissue specimen can be done by: [AI PGMEE 10]**
    a. ELISA (Enzyme Linked Immune Assay)
    b. Yolk sac inoculation
    c. Direct Immunofluorescence Antibody test (DFA)
    d. Polymerase Chain Reaction (PCR)

33. **Which of the following statement is not true regarding Chlamydia Trachomatis? [AI 2012]**
    a. Elementary body is metabolically active
    b. It is biphasic
    c. Reticulate body divides by binary fission
    d. Inside the cell it evades phagolysome

34. **A male patient with symptoms of urethritis. Examination reveals only pus cells without any organism. Most likely cause is: [AI 08; AIIMS 07]**
    a. *Chlamydia trachomatis*
    b. *H. ducreyi*
    c. *Treponema pallidum*
    d. *M. tuberculi*

35. **Most sensitive test for diagnosing chlamydia:**
    a. Gram's staining
    b. Nucleic acid amplification testing
    c. Elis A
    d. Fluoresant microscopy

Explanations and References with Illustrative Answers

1. **Ans. (b) Q fever** *Ref. Ananthnarayan 8/e, p 410, 9/e, p 411*

   "Q fever is air-borne disease."

   Lice act as vector in following diseases:

Disease	Causative agent
Epidemic typhus	R. prowazekii
Relapsing fever	Borrelia recurrentis
Trench fever	Rochalimaea quintana
Dermatitis	
Pediculosis	

   Note: Relapsing fever can be tick-borne also.

2. **Ans. (c) Adult female feeds on vertebrate hosts** *Ref. Ananthnarayan 8/e 408, 9/e, p 408; Harrison 18/e, p 1413, 19/e, p 1159*

   "Mite feeds on serum of warm blood animals only during there larval stage (chiggers) and adult mites feed only on plants"

   Scrub typhus
   - Caused by *O. tsutsugamushi*
   - **Transmitted** by *trombiculid mite* which also shows transovarian spread. It must be noted that disease is transmitted by chiggers (larva), not by adult mite
   - **Clinical features:** – Fever, headache, myalgia, cough and GI symptoms.
     – Classic case present with an eschar, regional lymphadenopathy and a maculopapular rash.
   - **Diagnosis:** Serologic assays (IFA, indirect immunoperoxidase and enzyme immunoassays) are main stay of diagnosis.

   Treatment – Rifampin
   – Azithromycin and clarithromycin

   Truely speaking doxycycline is not drug of choice but it can be used for all rickettsial infections.

3. **Ans. (c) *Rickettsia prowazekii*** *Ref. Ananthnarayan 8/e, p 406, 9/e, p 407; Park; 22/e, p 276*

   Most important point in the given question is "louse-borne rickettsial infection" which is only one i.e., Epidemic typhus which is caused by *R. prowazekii.*

   Epidemic Typhus
   - Also called as louse-borne typhus or classical typhus or Gaol fever.
   - Usually seen among military and refugee populations and famine areas.
   - Humans are only natural vertebrate host.
   - **Vector:** Body or head louse (*Pediculus humanus*) not pubic louse.
   - **Causative agent:** *R. prowazekii*
   - **Route of transmission:** Louse feces is rubbed over abraded skin. So, infection is transmitted from man-to-man by infected louse.
   - **Incubation period**: 5 to 15 days
   - **Clinical feature:** Fever, chills, rash (starts on trunk and spread over limbs sparing face, palms and soles), stupor and delirium.
   - Rickettsia may become latent in lymphoid tissue causing recrudescent typhus or Brill-Zinsser disease.

   Remember: Trench fever is also transmitted by louse but causative agent is Rochalimaea or Bartonella quintana which is excluded from Rickettsiacea, because it can grow in cell free media.

4. **Ans. (d) Weil-Felix reactions is very useful for diagnosis** *Ref. Jawetz 27/e, p 342; Harrison 17/e, p 1066, 18/e, p 1416, 19/e, p 1169*

   Weil Felix reaction is negative in Q fever, R pox, Trench fever.

   - Diagnosis of Q fever is made by indirect immunofluoroscence.

Clinical features of Q-fever:
- Headache
- Fatigue
- *No rash.*
- Fever
- Interstitial pneumonia

Rashes are seen in:
- Epidemic typhus (no eshar)
- Endemic typhus (no eschar)
- Scrub typhus (50% have eschar)
- RMSF (no eschar)
- Rickettsial pox (eschar)
- Fever boutonneuse (tache noire eschar).

Remember: Bacterial Zoonotic diseases:
- Q fever
- Anthrax
- Brucellosis
- Leptospirosis
- TB
- Plague

5. **Ans. (b) Is transmitted by bite of flea** *Ref. Ananthnarayan 8/e, p 407, 9/e, p 407; Park 22/e, p 274*

Endemic typhus or Murine or fleaborne typhus
- Caused by *R. typhi* or *R. mooseri*. It is zoonotic disease.
- No direct man-to-man transmission occur.
- Human acquire infection usually when saliva or feces of infected flea is rubbed over skin.
- Clinical features is similar to epidemic typhus in milder form.
- Its mammalian reservoir is rodents.
- Serology is ***diagnostic test.***
- Human infection is dead end infection.

Remember: Rickettsia cannot grow in artificial culture media.

6. **Ans. (a) (b) (d) (e) i.e. Zoonotic, Positive, Eschar, Spread** *Ref. Ananthnarayan 8/e, p 408-410, 9/e, p 407, 410*

Disease	Agglutination pattern		
	OX 19	OX 2	OX K
Epidemic typhus	+++	+	–
Brill-Zinsser disease	– –/+		–
Endemic typhus	+++	±	–
Tickborne spotted fever	++	++	–
Scrub typhus	–	–	+++

7. **Ans. (b) Trombiculid mite** *Ref. Harrison 17/e, p 1064, 18/e, p 1413*

Already explained

8. **Ans. (b) Flea is a vector of the disease** *Ref. Ananthnarayan 8/e, p 406, 7/e, p 414*

Already explained

9. **Ans. (a) *R. rickettsii*** *Ref. Park 22/e, p 273*

Already explained

10. **Ans. (c) Inhalation of aerosol** *Ref. Harrison 19/e, p 1161, 18/e, p 1415; Jawetz 27/e, p 347*

"Q fever is transmitted by inhalation of dust contaminated with rickettsia from placenta, dried feces, urine or milk or aerosols in slaughter houses."

Q fever:
- Caused by *Coxiella burnetii*
- A ***zoonotic*** disease
- Primary source of human infection are cattle, sheep and goats

- **Mode of transmission:**

In infected female animal, *C. burnetii* localizes uterus
↓
Infection reactivates during pregnancy
↓
High concentration of *Coxiella* in placenta
↓
Soil gets contaminated during parturation
↓
Coxiella aerosols generates during storms
↓
Human infection

- **Diagnosis:**
  - Mainly serological through microagglutination, compliment fixation.
  - Isolation is possible but is not recommended due to hazard of laboratory infection.

Remember: Q fever: – Gives negative Weil-Felix reaction.
– Also transmitted by infected milk.

11. **Ans. (d) Epidemic typhus** *Ref. Park 22/e, p 273*
Already explained

12. **Ans. is (d) Trombiculid mite** *Ref. Ananthnarayan 8/e, p 406, 9/e, p 406; Park 22/e p 712*
Trombiculid mite transmit both rickettsia disease (Rickettsial pox) and orientea disease (scrub typhus)

Arthropod-borne diseases	
Arthropod	***Disease transmitted***
Mosquito	Malaria, filaria, viral encephalitis (e.g. Japanese encephalitis), viral fevers (e.g. dengue, West Nile, viral haemorrhagic fevers (e.g. yellow fever, dengue haemorrhagic fever)
Housefly	Typhoid and paratyphoid fever, diarrhoea, dysentery, cholera, gastroenteritis, amoebiasis, helminthic infestations, poliomyelitis, conjunctivitis, trachomas, anthrax, yaws, etc.
Sandfly	**Kala-azar**, oriental sore sandfly fever, oraya fever
Tsetse fly	Sleeping sickness
Louse	Epidemic typhus, relapsing fever, trench fever, pediculosis
Rat flea	Bubonic plague, endemic typhus, chiggerosis, hymenolepis diminuata
Blackfly	Onchocerciasis
Reduviid bug	Chagas disease
Hard tick	Tick typhus, viral encephalitis, viral fevers, viral haemorrhagic fever, (e.g. Kyasanur forest disease), tularemia, tick paralysis, human babesiosis
Soft tick	Q fever, relapsing fever Mnemonic: **QRST**
Trombiculid mite	Scrub typhus, Rickettsial-pox
Itch mite	Scabies
Cyclops	Guinea-worm disease, fish tapeworm (D. latus)
Cockroaches	Enteric pathogens

13. **Ans. (b) Rocky mountain ...** *Ref. Harrison 18/e, p 1407, 19/e, p 1154*
Family rickettsiae includes six genera:
- *Rickettsia*
- *Orientia*
- *Neorickettisa*
- *Ehrlichia*
- *Anaplasma*
- *Coxiella*

As question is about disease by rickettsia answer is RMSF (which is caused by *Rickettsia rickettsii*) only as scrub typhus is caused by *Orientia tsutsugamushi.*

14. **Ans is (a) crimean... and (b) Rock...** *Ref. Park 22/e p 712*
Crimean congo is a viral illness, caused by flavi virus and is transmitted by ticks
For full details see previous answers

15. **Ans (d) Penicillin is the treatment** *Ref. Parson 20/e, p 1070, 1074*
Penicillin is not effective against Chlamydia

Drugs effective against *Chlamydia* include
- Tetracycline
- Azithromycin
- Rifampicin
- Erythromycin
- Sulfonamides

- *Chlamydia* can be cultured, but not in cell free media.
- In most cases trachoma inflammation undergoes spontaneous resolution and only few cases progress to conjunctival scarring.

16. Ans. (a) McCoy cell line *Ref. Ananthnarayan 8/e, p 419-420, 9/e, p 417-418; Jawetz 27/e, p 354*

"Complaint of urethritis with no result on gram staining signifies nongonococcal urethritis."

MC cause of NGU is *Chlamydia trachomatis* and urethritis is one of the commonest manifestation of genital chlamydiasis.

Diagnosis of genital chlamydiasis (D-K serovars)

I. Culture:
- Scarpe epithelial cells from 1-2 cm deep into endocervix.
- Dacron, cotton, rayon or calcium alginate on plastic shaft should be used to collect specimen.
- Inoculum is centrifuged onto the monolayer of cyclohexinide treated McCoy cells.
- This is incubated at 35-37°C for 48-72 hours.
- Monolayers can be increased.
- Examine monolayers by direct IF to see inclusion bodies.
- This method is 80% sensitive but 100% specific.

Remember: HeLa cell culture can also be used.

II. Direct cytologic examination (direct fluorescent antibody) and enzyme-linked immunoassay.

III. Nucleic acid detection by PCR and LCR are test of choice to diagnose genital *C. trachomatis* infections.

IV. Serology –Serum antibodies are more common than trachoma because of more antigenicity in genital tract.

Remember: LJ medium is for *Mycobacteria tuberculosis*. Thayer Martin medium is for Neiserria.

17. Ans. (b) Elementary body is metabolically active *Ref. Ananthnarayan 8/e, p 416, 9/e, p 416; Jawetz 27/e, p 351*

Reproductive Cycle of Chlamydiae

Elementary body (EB)
- Stable spherical form, metabolically inactive
- Extracellular phase
- Infectious form
- 200 - 300 nm diameter
- Rigid trilaminar cell wall
- Electron dense nucleoid (contains DNA)
- DNA = RNA

↓

Attach to surface of susceptible epithelial cell near base of microvilli by adhesins (e.g. major outer membrane protein); receptors (heparin sulfate like proteoglycans in case of *C. trachomatis*)

↓

Engulfment of EB into host cell either by endocytosis into clathrin-coated pits or pinocytosis into non-coated pits. Lysosomal fusion is inhibited by unknown mechanism, so EB form of chlamydiae is protected under membrane bound environment.

↓

Reorganisation of EB by spheroplast-like transformation/loss of cross-linking of EB membrane proteins.

↓

Reticulate body (Initial body form)
- Pleomorphic stage
- Intracellular form
- Growing and replicative form
- 500-1000 nm size
- No electron dense nucleoid
- RNA > DNA

Within membrane bound vacoule RB divides by binary fission repeatedly to form EB.

↓

Cytoplasmic inclusion bodies form (EB filled vacoule)

↓

This EB liberate from host cell to infect new cells.

18. Ans. (d) Community acquired pneumonia *Ref. Harrison 18/e, p 1422-1425, 19/e, p 1172; Ananthnarayan 8/e, p 417, 9/e, p 416*

Human disease caused by Chlamydiae		
Species	**Serotype**	**Disease**
C. trachomatis	A, B, Ba, C	Endemic blinding trachoma
C. trachomatis	D - K	Inclusion conjunctivitis, genital chlamydiasis, infant pneumonia
C. trachomatis	L1, L2, L3	Lymphogranuloma venereum
C. psittaci	Many serotype	Psittacosis
C. pneumoniae	Only one serotype	Acute respiratory disease

Remember: Inclusion conjunctivitis include inclusion blenorrhea or ophthalmia—neonatorum and swimming pool conjunctivitis.
C.pneumoniae is associated with 10% of cases of community acquired pneumonia

Remember: ***Four most common cause of community acquired pneumonia are:***
- S. pneumoniae
- H. influenzae
- Chlamydia pneumoniae
- Legionella.

19. Ans. (d) The group specific antigen is responsible for the production of complement fixing antibodies *Ref. Jawetz 27/e, p 355; Ananthnarayan 8/e, p 416, 9/e, p 418*

"Genus or Group specific heat stable LPS antigen is responsible for CFT while serovar specific major membrane protein is responsible for microimmunofluorescence."

Characteristic of Chlamydiae			
Feature	**C. trachomatis**	**C. pneumoniae**	**C psittaci**
Inclusion morphology	Round, vacuolar	Round, dense	Large, variable shape, dense
Glycogen in inclusions	Yes	No	No
Elementary body morphology	Round	Pear-shaped, round	Round
Susceptible to sulfonamides	Yes	No	No
DNA homology to C pneumoniae	<10%	100%	<10%
Plasmid	Yes	No	Yes
Serovars	15	1	> 4
Natural host	Humans	Humans	Birds
Mode of transmission	Person-to-person, mother-to-infant	Airborne person-to-person	Airborne bird excreta to humans
Major diseases	Trachoma, STDs, infants pneumonia, LGV	Pneumonia, bronchitis, sinusitis	Psittacosis, pneumonia, fever of unexplained origin

20. Ans. (c) Polymerase chain reaction *Ref. Harrison 18/e, p 1426, 19/e, p 1170*

Nucleic Acid Amplification ***"Amplification assays such as ligase chain reaction and polymerase chain reaction are the most sensitive chlamydial diagnostic method available."***

Diagnostic methods for Chlamydia	Accuracy
Cell culture technique	Low sensitivity (60 to 80%)
Direct immunofluorescent antibody test	70-80% sensitive and quite specific
ELISA	60-80% sensitive
LCR and PCR	Most sensitive method available

Note: For occular disease nucleic acid amplification test (NAAT) are not approved by FDA.
For genital chlamydiasis NAAT specimen can be:
- Urine in males
- Cervical/vaginal swabs in females

21. Ans. (a) Q. fever *Ref. Ananthnarayan 8/e, p 417, 9/e, p 411*

Already explained

22. **Ans. (b) Cause non-gonococcal urethritis** *Ref. Ananthnarayan 8/e, p 420, 9/e, p 421; Jawetz 27/e, p 358*

C. Psittaci

- Causes psittacoses in humans/birds, ornithosis in birds, meningopneumonitis, feline pneumonitis and other animal diseases.
- Form ***diffuse intracytoplasmic inclusions*** that lack glycogen, not stained by I_2 and not inhibited by sulphadiazine or cycloserine. Heat stable group reactive. CF antigen resist proteolytic enzymes so seems to be lipopolysaccharide.
- Psittacosis is disease of human acquired from contact with birds and also includes infection of psittacine birds.
- Ornithosis is infection in all types of domestic birds.
- Infection in birds is usually subclinical (carriers).
- ***Human infection*** is usually **occupational** as in poultry workers, pigeon farmers, pet-shop owners, bird fencers and veterinarian. Usually occur by inhalation of infected dried feces.
- ***Incubation period*** is about **10 days.**
- Manifest as influenza/atypical pneumonia/sepsis.
- Antibodies to species specific antigen can neutralize toxicity and infectivity.
- **Diagnosis:** – Culture is dangerous and if done; then isolation should be attempted only in special laboratories as laboratory infection is serious hazard.
  – *Detection of antigen by direct fluorescent antibody staining or by immunoassay or PCR is preferred,* serology (confirmatory) by CFT or MIF.
- **Treatment:** – DOC is tetracycline.
  – Should be continued for 10 days after defervescence to prevent relapse.

23. **Ans. (b) Culture on McCoy cells** *Ref. Ananthnarayan 8/e, p 420, 9/e, p 419*

"Genital chlamydiasis is suspected if Gram stained smear of urogenital exudates show significant number of neutrophils (> 4/oil immersion field in urethritis, > 30 in cervicitis) in absence of gonococcal bacteria."

McCoy and HeLa cell lines are the most common cell lines to cultivate chlamydia

24. **Ans. (b) Enzyme immunoassay** *Ref. Ananthnarayan 8/e, p 418, 9/e, p 418*

Isolation of Chlamydiae can be done by:

a. Inoculation into yolk sac of embryonated eggs of 6 - 8 day old chick embryo which may be pretreated by streptomycin or polymyxin B.
b. Inoculation into experimental animals (mice): Intranasal; intraperitoneal or intracerebral inoculation
c. Tissue/cell culture:
   - *Preferred mode*
   - Commonly used cell lines are McCoy and HeLa cells.
   - Cell cultures are pretreated by irradiation or chemicals such as 5-iodo-2 deoxyuridic or cycloheximide to enhance replication and detection of inclusion bodies.
   - Pretreatment with DEAE dextrax or centrifugation after inoculation promotes contact between chlamydiae particles and cell monolayer.

25. **Ans. (c) They can grow in cell free culture media** *Ref. Ananthnarayan 8/e, p 415, 9/e, p 415*

- *Chlamydia* are obligate intracellular parasite so, they cannot be grown in cell free media.
- McCoy and Hela cell lines are ***MC cell lines*** used to culture chlamydiae.
- Chlamydia are Gram-negative coccobacilli.
- Chlamydia do not have peptidoglycan cell wall.
- They lack enzymes of electron transport chain. So require ATP from host cells and are called energy parasites.
- ***Drugs effecitve against chlamydiae:*** Doxycycline azithromycin, erythromycin.

Remember: Other obligate parasite: Rickettsiaceae, M. laprae, pathogenic treponemes and Coxiella burnetii.

26. **Ans. (a)** ***Chlamydia trachomatis*** *Ref. Harrison 19/e, p 1167; Ananthnarayan 9/e, p 420; 8/e 230*

"Complaint of urethritis with no result on Gram staining signifies Non-gonoccoccal urethritis."

27. **Ans. (d) Urethritis** *Ref. Ananthnarayan 8/e, p 419, 7/e, p 424*

- Urethritis is one of the presentation of serovar D-K
- Genital chlamydiasis, caused by serotype D-K of *C. trachomatis.*
- *C. pneumonia* increase the risk of arthrosclerosis, asthma, sarcoidosis.

28. **Ans. (a), (c) and (d) Conjunctivitis, Polyarthritis, Mucosal lesions** *Ref. Harrison 19/e, p 1168, 18/e, p 2778*

Reiter's syndrome consists of conjunctivitis, urethritis, (or in female patient cervicitis) arthritis and characteristic mucocutaneous lesion.

- Pathogenesis is unknown. However more than 80% affected patient belong **to HLA-B-27.**
- It may develop in 1-2% cases of non-gonococcal urethritis and is thought to be the *most common type of peripheral inflammatory arthritis in young men.*
- Other implicated bacteria includes Salmonella, Shigella, Yersinia or Campylobacter
- Arthritis usually begin 4 weeks after the onset of urethritis
- *Knees followed by ankle are the most frequently involved joint*

29. **Ans. (a), (b) and (c) HeLa; HeP2; and McCoy** *Ref. Ananthnarayan 8/e, p 418, 9/e, p 418*

Cell lines for chlamydiae are:

- McCoy
- HeLa
- HEp - 2,
- HL.

Human diploid fibroblast series and vero cell lines are used for virus isolation.

30. **Ans. (a) and (c) Infertility and pelvic inflammatory disease** *Ref. Harrison 19/e, p 1167-1170, 18/e, p 1422-1425*

Chlamydial diseases are:

- **In men** – NGU, epididymitis
- **In women** – Cervicitis, salpingitis, urethritis, PID, infertility
- **Either sex** – Proctitis, LGV, Reiter syndrome
- **Neonates** (As a result of perinatal infection) – Conjunctivitis, pneumonia

31. **Ans. (b) Nucleic acid amplification test** *Ref. Harrison 17/e, p 1072, 1074, 1075, 18/e, p 1426, 19/e, 1171*

"At least 1/3 of males with C. trachomatis urethral infection have no symptoms. *Use of NAAT(Nucleic acid amplification test) using PCR, LCR,TMA on first void urine specimen is highly sensitive in detecting these infections".*

Among all test for detection of chlamydia these NAAT tests are most sensitive. They can be applied for all infection sites viz conjunctiva (on conjunctival scrapings); pneumonia (on sputum); proctitis (rectal secretions) etc.

Types of NAAT for Chlamydia

- PCR (Polymerase chain reaction)
- LCR (Ligase chain reaction)
- TMA (Transcription mediated assay)
- Gene probe optima CT assay

- In all NAAT the ***Gen Probe optima CT assay*** which utilises target capture and RNA amplification is *most sensitive and specific.*
- The main limitation of these tests is their cost and availability

32. **Ans. (b) Yolk Sac inoculation** *Ref. Ananthnarayan 8/e, p 418, 9/e, p 418*

Already explained

33. **Ans. (a) Elimentary body is** *Ref. Ananthnarayan 8/e, p 415, 9/e, p 416*

Already explained

34. **Ans. (a)** ***Chlamydia trachomatis*** *Ref. Harrison 17/e, p 1072, 18/e, p 1423, 19/e, p 1167*

The patient is suffering from non-gonococcal urethritis and *C. trachomatis is the most common cause of non gonococcal urethritis.*

Cause of non-gonococcal urethritis	
Chlamydia trachomatis (MC)	Ureoplasma urealyticum
Trichomonas vaginalis	Herpes simplex virus
Mycoplasma hominis	CMV
Gardnerella vaginalis	*Acinetobacter lwoffi, Ac calcoaceticus*
Candida albicans	

35. **Ans. (b) Nucleic acid amplification testing** *Ref. Harisson 18/e, p1426*

Already explained

Chapter Review

1. The primary site of multiplication of rickettsial organisms is in the: [AI 90]
a. Parenchymal cells of the liver
b. Endothelial cells of small vessels
c. Media of arteries
d. Adventitia of all blood vessels
[Ref. Ananthanarayan 8/e, p 406, 9/e, p 406]

On entry into the human body, the rickettsiae multiply locally and enter the blood. They become localized in vascular endothelial cells which gets enlarged, degenerate and cause thrombus formation with partial or complete occlusion of vascular lumen.

2. Weil Felix reaction with OXK antigen indicates infection with: [AIIMS 92]
a. *R. tsutsugamushi* b. *R. moseri*
c. *R. quintana* d. *R. akari*
[Ref. Ananthanarayan 8/e, p 406, 9/e, p 410]

3. Mite transmits: [AI 91]
a. Scrub typhus b. Trench fever
c. Endemic typhus d. Epidemic typhus
[Ref. Ananthanarayan 8/e, p 410, 9/e, p 408]

4. Anthropozoonosis are all except: [AI 93]
a. Guinea worm infection
b. Rabies
c. Plague
d. Hydatid cyst *[Ref. Park 22/e, p 90]*

- **Anthropozoonoses:** Infections transmitted to non from vertebrate animal e.g. Rabies, plague
- **Zoonthroposis:** Infections transmitted from non to bertebrate animal e.g. human tuberculosis in cattle.

5. Most common diagnostic test in LGV is: [PGI 93]
a. Fluorescent antibody
b. Complement fixation test
c. Cell culture
d. Frei's test
[Ref. Ananthanarayan 8/e, p 420, 9/e, p 421]

6. *Chlamydia* cause all of the following except: [PGI 93]
a. Trachoma b. Non-gonococcal urethritis
c. Pneumonia d. Parotitis
[Ref. Ananthanarayan 8/e, p 417, 230, 9/e, p 420, 421]

7. *Chlamydia trachomatis* infection causes the following except: [Comed 07]
a. Pneumonitis b. Rhinitis
c. Conjunctivitis d. Urethritis
[Ref. Anantharayan 8/e, p 419, 9/e, p 426]

8. *Chlamydia trachomatis* causes all except: [UP 08]
a. Ophthalmia neonatorum
b. Lymphogranuloma venereum
c. Q-fever
d. Trachoma *[Ref. Anantharayan 8/e, p 417, 9/e, p 420,411]*

9. Which of the following is an obligate parasite? [AI 98]
a. *Mycoplasma* b. *Chlamydia trachomatis*
c. Gram -ve bacilli d. Gram +ve cocci
[Ref. Anantharayan 8/e, p 405, 9/e, p 416]

10. Transovarian transmission is seen in: [DNB 2013]
a. Ricketssiae b. Chlamydia
c. Both d. None
[Ref. Anantharayan and Paniker 9/e, p 408; Park 22/e 94]

11. Neil Mooser's reaction is given by: [TN 02]
a. Rickettsial infection b. Chlamydial infection
c. *Mycoplasma* d. Pnemococcai infection
[Ref. Ananthnarayan 8/e, p 407, 9/e, p 407]

12. Microorganism that does not obey Koch's postulates: [Kerala 02, AI 89]
a. *M. tuberculosis* b. Poliovirus
c. *M. leprae* d. *Streptococcus*
[Ref. Ananthnarayan 8/e, p 405, 9/e, p 4]

Koch's postulates are not valid for microorganism that can't be grown in all free cutters media e.g. M. leprae, chlamydia, many virus.

Now Koch's postulate largely have been suplanted by Bradford Hill criteria of infectious disease

13. *Rickettsia prowazekii* is transmitted by: [SRMC 02]
a. Fleas b. Mites
c. Tick d. Louse
[Ref. Ananthnarayan 8/e, p 406, 9/e, p 407]

14. All the following statement are true about rickettsial excepts: [UPSC 02]
a. These are transmitted by arthropod vectors
b. Eschar is not seen in RMSF
c. Well Felex reaction may be diagnostic
d. Cephalosporins are drug of choice
[Ref. Ananthnarayan 8/e, p 408, Harrison 18/e, p 1410]

Tetracyclines are the first choice drug in Rickettsial infections with "Chloramphenicol as an alternative"

Answers 1. b. Endothelial ... 2. a. *R. tsutsugamushi* 3. a. Scrub typhus 4. a. Guinea worm ... 5. c. Cell culture
6. d. Parotitis 7. b. Rhinitis 8. c. Q-fever 9. b. Chlamydia... 10. a. Ricketssiae
11. a. Rickettsial ... 12. c. *M. leprae* 13. d. Louse 14. d. Cephalosporins ...

15. Chlamydia trachomatis, the causative agent for trachoma:
a. Is a yeast **[Kar 2003]**
b. Is an intracellular organism
c. Forms extracellular bodies which are diagnostic
d. Is never demonstrable in conjunctival scrapings
[Ref. Ananthnarayan 8/e, p 415, 417, 9/e, p 416, 418]

16. Causative agent of Q fever: **[Kolkata 03]**
a. *R. quintana* b. *R. prowazekii*
c. *R. typhi* d. *Coxiella burnetii*
[Ref. Ananthnarayan 8/e, p 410, 9/e, p 411]

17. Reiter's syndrome is due to: **[Jharkhand 04]**
a. *Chlamydia* b. *Neisseria*
c. *Mycoplasma* d. Herpes virus
[Ref. Ananthnarayan 9/e, p 420, 8/e, p 419]

- Reiter's syndrome is a triad of conjunctivitis, urethritis or cervicitis and polyarthritis.

Mnemonic: CUP

- Riter's disease is severe form of staphylo-coccal scalded skin syndrome (SSSS)
- Fitz Hugh Curtis syndrome (= Perihepatitis) is also caused by *C. trachomatis* (serotypes D to K).

18. For all of the following reaction Well Felix reaction is diagnostic except: **[SGPGI 05]**
a. Endemic typhus b. Scrub typhus
c. Epidemic typhus d. Q fever
[Ref. Ananthanarayan 8/e, p 410, 9/e, p 410]

19. Endemic typhus is transmitted by: **[DNB 05]**
a. Rat flea b. Mite
c. Tick d. None
[Ref. Ananthanarayan 8/e, p 406, 9/e, p 407]

20. All of the following can be grown in cell free culture media except: **[SGPGI 05, DNB 09]**
a. *Mycoplasma* b. *Rickettsia*
c. *Salmonella* d. *Campylobacter*
[Ref. Ananthnarayan 8/e, p 405, 9/e, p 406, 412]

21. Which rickettsiae are able to grow in cell free media? **[Jharkhand 05]**
a. *R. quintana* b. *R. rickettsii*
c. *R. typhi* d. *R. Tsuisugamushi*
[Ref. Anantharayan 8/e, p 404, 9/e, p 412]

22. Vascular endothelial infection is caused by: **[DNB 2013]**
a. Rickettsiae
b. Mycoplasma
c. Chlamydia
d. None *[Ref. Anantharayan and Paniker 9/e, p 407]*

Transovarian spread occurs in R ricketssiae

23. All are true about *Rickettsia* except: **[AIIMS 99]**
a. Obligate intracellular
b. Gram +ve bacillus
c. Arthropods are vector
d. Weil Felix test used in diagnosis
[Ref. Anantharayan 8/e, p 404, 9/e, p 406]

24. Which is not transmitted by arthropod? **[AIIMS 97]**
a. *Rickettsia prowazakii*
b. *Coxiella burnetil*
c. *Rickettsia akari*
d. *Rickettsia rickettsi* *[Ref. Park 22/e, p 273]*

25. Neill-Mooser reaction is used to diagnose: **[PGI 99]**
a. Rickettsiae
b. Chlamydiae
c. *Mycoplasma*
d. Herpes
[Ref. Anantharayan 8/e, p 405, 9/e, p 407]

Neill-Mooser reaction

- Male guinea pigs are inoculated intraperitoneally with blood from a case of endemic typhus or culture of *R. typhi*, they develop fever and characteristic scrotal inflammation. This is called *Neill - Mooser reaction.*
- This is ***negative*** with R. prowazaki but ***positive*** in *R. typhi*, *R.conori* and *R.akari.*

Answers **15.** b. Is an intra... **16.** d. *Coxiella* ... **17.** a. *Chlamydia* **18.** d. Q fever **19.** a. Rat flea
20. b. *Rickettsia* **21.** a. *R. quintana* **22.** a. Rickettsiae **23.** b. Gram-positive **24.** b. Coxiella...
25. a. *Rickettsia*

NEET Pattern Questions

1. Inclusion body is seen in:
a. Rickettsiae b. Chlamydia
c. Mycoplasma d. H. Pylori
[Ref. Ananthanarayan, 8/e, p 415, 9/e, p 416]

2. All of the following are true about Chlamydia except:
a. Gram positive
b. Causes trachoma
c. Causative organism of psittacosis
d. Are also called tasophilic viruses
[Ref. Ananthanarayan, 9/e, p 416]

3. True about chlamydia are all except:
a. Obligate intracellular organism
b. Gram positive
c. Reticulate body is metabolically active
d. Replicate by binary fission
[Ref. Ananthanarayan 9/e p 415]

- Cell wall of chlamydia resemble Gr (–)ve bacteria.

4. Obligatory intracellular organism is:
a. Mycoplasma b. Chlamydae
c. Cryptococcus d. H. pylori

5. Well Felix reaction for Scrub typhus shows positivity for:
a. OXK b. OXK + OX19
c. OX-2 d. OX-19
[Ref. Ananthanarayan, 9/e, p410]

6. Which of the following is used for Rickettsia?
a. Well-Felix reaction b. Rose-waler test
c. Poul-Bunnel test d. VDRL

7. Bartonella henselae causes all except:
a. Oraya fever
b. Cat scratch disease
c. Bacillary angiomatosis
d. SABE

- Bartonella are tiny gram negative bacteria, usually transmitted by arthropods which invades mammalian endothelial cells and blood cells. Human pathogenic strains are:
B. Baciliformis, B. quintana and B. henslae

Organism	*Disease*
B. Henslae	Cat scratch disease, bacillary angiomatosis bacillary peliosis, bacterial endocarditis.
B. quintana	Trench fever or five day fever
B. bacilliformis	Oraya fever, Verruga peruana (carrions disease)

8. Inclusion body is seen in:
a. Rickettsiae b. Chlamydia
c. Mycoplasma d. H. pylori
[Ref. Ananthanarayan, 9/e, p 419]

9. LGV (lymphogranuloma venerum) is caused by:
a. Treponema pallidum
b. Chlamydia trachomatis
c. Calymmatobacter granulomatosis
d. H.ducrevi *[Ref. Harrison, 19/e, p 1169]*

10. How does chlamydia differ from other usual bacteria?
a. Lack cell wall
b. Cannot grow in cell free culture media
c. Contains inclusion body
d. None of the above *[Ref. Ananthanarayan, 9/e, p 415]*

Cell wall of chlamydia resemble that of Gr(-)ve bacteria.

11. Frie's test is useful for diagnosis of:
a. Mycoplasma b. Rickettsia
c. Sarcoidosis d. Chlamydia
[Ref. Ananthanarayan, 9/e, p 421]

- **Frie test:** Demonstration of hypersensitivity by skin testing was widely used for diagnosis of LGV. But due to high false positivity it has been given up.

12. True about chlamydia:
a. Extracellular bacteria
b. HeLa cells for isolation
c. Gram positive *[Ref. Ananthanarayan, 9/e, p 419]*
d. Penicillin is drug of choice

13. Tunica reaction is positive in:
a. R prowazekii b. R typhi
c. R tsutsugamushi d. R akari
[Ref. Ananthanarayan, 9/e, p 407]

- **Neil-Mooser = Tunica reaction:** When male guinea pigs are inoculated intraperitoneally with blood from a case of endemic typhus or with culture of R.typhi, they develop fever and a characteristic scrotal inflammation. The testes can not be pushed back into scrotum because of inflammatory adhesions between the layers of tunica vaginalis. The reaction is negative with R. prowazakaii.

14. Indian tick typhus is caused by:
a. R typhi b. R conorii
c. R akari d. C burnetii
[Ref. Park, 22/e, p 275]

Answers 1. b. Chlamydia 2. a. Gram positive 3. b. Gram positive 4. b. Chlamydae 5. a. OXK
6. a. Well-Felix reaction 7. a. Oraya fever 8. b. Chlamydia 9. b. Chlamydia 10. b. Cannot grow...
11. d. Chlamydia 12. b. HeLa cells 13. b. R typhi 14. b. R conorii

15. Weil-Felix reaction is heterphile antibodies reaction due sharing of Rickettsial antigen with:
a. Shigella
b. Proteus
c. Chlamydia
d. Mycoplasma *[Ref. Ananthanarayan, 9/e, p 410]*

16. Rickettsial disease with positive Weil-Fellix reaction of only OX-19:
a. Epidemic typhus
b. Scrub thphus
c. Trench fever
d. Q-fever *[Ref. Ananthanarayan, 9/e, p 410]*

17. Ricketsial infections cause 30% mortality due to:
a. Endothelial injury
b. Hemodynamic instability
c. Endocarditis
d. Renal failure *[Ref. Ananthanarayan, 9/e, p 407]*

18. "Genital elephantiasis" is seen in:
a. Rickettsia
b. Chancroid
c. Lymphogranuloma venereum
d. Syphilis *[Ref. Anantharayan 8/e, p 420, 9/e, p 420]*

In tertiary stage of LGV elephentiasis of vulva is there

19. Trachoma is caused by which serotype of chlamydia trachomatis?
a. D to K
b. A, B, C
c. L_1 L_2 L_3
d. All of the above
[Ref. Ananthanarayan, 9/e, p 416]

20. Chlamydia pneumoniae causes:
a. LGV
b. Atherosclerosis
c. Inclusion conjunctivitis
d. Trachoma *[Ref. Ananthanarayan, 9/e, p 422]*

21. True about chlamydia is:
a. Replicative form is elementary body
b. Infective form to host cell is elementary body
c. Cell wall contains N-acetylmuramic acid and peptidoglycan
d. All of the above are correct
[Ref. Ananthanarayan, 9/e, p 416]

22. Cat scratch disease is caused by:
a. Streptobacillus moniliformis
b. Spirillum minus
c. B. Henselae
d. R. tsutsugamushi *[Ref. Ananthanarayan, 9/e, p 414]*

Disease caused by bartonella henselae
- Cat scratch desease
- Bacillary angiomatosis
- Bacillary peliosis

23. Bartonella henselae causes which disease?
a. Bacillary peliosis
b. Chancre redux
c. Rat bite fever
d. Spirillum fever
[Ref. Ananthanarayan, 9/e, p 413]

24. Bartonella quintana causes:
a. Trench fever b. Scrub typhus
c. Endemic typhus d. Epidemic typhus
[Ref. Ananthanarayan, 9/e, p 412]

25. Vector for scrub typhus:
a. Reduvid bug b. Trombiculid mite
c. Enteric pathogens d. cyclops
[Ref. Ananthanarayan, 9/e, p 406]

26. Endemic typhus is caused by:
a. R prowazekii b. R typhi
c. R tsutsugamushi d. R akari
[Ref. Ananthanarayan, 9/e, p 406]

27. Weil-Felix reaction positive with OXK antigen in:
a. Trench fever
b. Scrub typhus
c. Endemic typhus
d. Epidemic typhus
[Ref. Ananthanarayan, 9/e, p 410]

See answer no. 6 in explanatory answers

28. What is trench fever:
a. Q-fever
b. 5-days fever
c. Boutonneuse fever
d. Indian tick typhus
[Ref. Ananthanarayan, 9/e, p 412]

Trench fever or five day fever is an exclusive human disease seen in European soldiers fighting in trenches.
- Transmitted by body louse. Body louse remain infective for whole life
- Etiologic agent is Rochalimae quintana also called as B. quintana

29. Brill-Zinsser disease is:
a. Recrudescent of R prowazekii infection
b. Recrudescent of R typhi infection
c. Recrudescent of R conorii infection
[Ref. Ananthanarayan, 9/e, p 407]

Answers **15.** b. Proteus **16.** a. Epidemic typhus **17.** a. Endothelial injury **18.** c. Lympho... **19.** b. A, B, C
20. b. Atherosclerosis **21.** b. Infective form... **22.** c. B. Henselae **23.** a. Bacillary peliosis **24.** a. Trench fever
25. b. Trombiculid... **26.** b. R typhi **27.** b. Scrub typhus **28.** b. 5-days fever **29.** b. Recrudescent...

30. **Which of the following infection is mainly diagnosed by serological tests?**
    a. Actinomycosis
    b. Q Fever
    c. TB
    d. Leprosy *[Ref. Ananthanarayan, 9/e, p 412]*

31. **Chlamydia is associated with which bodies?**
    a. Torres bodies
    b. Negri bodies
    c. Reticulate bodies
    d. Bollinger bodies

32. **Painful matted suppurative lymphadenitis after healing of genital lesion is seen in:**
    a. Syphilis
    b. Chancroid
    c. LGV
    d. Donovanosis *[Ref. Ananthanarayan, 9/e, p 418]*

33. **True about chlamydia:**
    a. Extracellular bacteria
    b. HeLa cells for isolation
    c. Gram positive
    d. Penicillin is drug of choice
    [Ref. Ananthanarayan, 9/e, p 418]

34. **Eschar is seen in all the Rickettsial diseases excent:**
    a. Scrub typhus
    b. Rickettsial pox
    c. Indian tick typhus
    d. Endemic typhus *[Ref. Jawetz 27/e, p 343]*

35. **Bubus form is which stage of LGV:**
    a. Prmary
    b. Secondary
    c. Tertiary
    d. Latent
    [Ref. Harrison 19/e, p 1170, Greenwood 18/e, p 385]

Answers 30. b. Q Fever 31. c. Reticulate bodies 32. c. LGV 33. b. HeLa cells for isolation
34. d. Endemic typhus 35. b. Secondary

CHAPTER

22

Spirochetes

Group comprising: Elongated, motile, flexible bacteria.

Characteristic feature of spirochetes is *presence of varying number of* ***endoflagella*** which are polar flagella situated between outer membrane and cell wall (periplasmic space). Unlike flagella of other bacteria they *don't protrude outside.*

Pathogenic spirochetes belong to genera:

1. *Treponema*
2. *Borrelia*
3. *Leptospira*

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Treponema

- Spiral bacteria actively motile (by endoflagella)
- Don't stain by Gram's method
- Possess fine cytoplasmic filaments which are absent in Borrelia
- Pathogenic treponemes cannot be cultivated.

TREPONEMA

- Relatively short slender spirochetes with fine spiral and **pointed or rounded ends.**
- Pathogenic treponemes **have not been successfully cultivated in** cell free media while the non-pathogenic [which are commensals] can be cultivated.
- **Pathogenic treponemes include:**
  - ***T. pallidum*** (causative agent of endemic and venereal syphilis)
  - ***T. partenue*** (causative agent of yaws)
  - ***T. carateum*** (causative agent of pinta)

  They are identical in their morphology, antigenic structure and other biochemical features, differs only in clinical feature of disease they produce. But recently molecular signatures have been identified that can differentiate the three subspecies of *T. pallidum* by PCR based methods. *... Harrison 19/e, p 1133, 18/e, p 1380*

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- ***T. pallidum:*** Syphilis
- ***T. partenue:*** Yaws
- ***T. carateum:*** Pinta

TREPONEMA PALLIDUM

Morphology

- A thin spiral organism which is actively motile through endoflagella.
- *Seen* by **immunofluorescence staining** or dark field illumination or phase contrast microscope.
- *Stained* by **silver impregnation methods.** Fontana method useful for staining films and Levaditi method for tissue sections.

Cultures and Growth

- **Pathogenic treponemes** have **never been cultured** continuously on artificial media, in fertile eggs or in tissue culture as pathogenic treponemes lack genes required for de novo synthesis of amino acids, nucleotides and lipids. They also lacks the genes encoding for the enzymes of Krebs cycle and oxidative phosphorylation
- *Reiter strain (T. phagedenis)* - Non-pathogenic. treponeme; shows morphological and antigenic similarities with *T. pallidum;* can grow in artificial culture.
- As such *T. pallidum* is ***microaerophilic*** organism and survives best in 1-4% O_2.
- Virulent *T. pallidum* strains can be maintained by serial testicular passage in rabbits. One such strains called **Nichol strain** was isolated in 1912 and is still being propagated.
- In whole blood or plasma stored at 4°C, organisms remain viable for at least 24 hours, which is of potential importance in blood transfusions.

Antigenic structure

- Treponemal infection induce 3 antibodies:
  - **Reagin antibody:** It is responsible for *Wasermann reaction, Kahn test* and *VDRL.* In these reactions a hapten called cardiolipin *[extracted from beef heart]* is used as antigen. Chemically cardiolipin is diphosphatidylglycerol.
  - ***Antibody to group antigen*** which is found in both pathogenic and non-pathogenic treponemes.
  - **Antibody to species specific** antigen which is polysaccharide in nature and is positive only with sera of patients infected with pathogenic treponemes.

Clinical Manifestations

Natural infection with *T. pallidum* occurs ***only in*** human beings. It causes:

1. **Venereal Syphilis**
   - Acquired by sexual contact. **Infectivity** of patient to its sexual partner is maximum during ***1st two years of disease.***
   - In 1905, *Schaudinn and Hoffmann* discovered Treponema pallidum in tissue of patients with syphilis. One year later, the first effective test for syphilis, the Wassermann test, was developed
   - Treponema rapidly penetrates intact mucous membrane or microscopic abrasions in skin and within few hours enters the lymphatics and blood to produce systematic infection.
   - Blood from the patient with incubating or early syphilis is infectious.
   - Natural history fall into 3 ***stages:***

Primary syphilis	Primary lesion of syphilis *is painless hard chancre* at the site of entry of spirochete which heal without scar in 10 - 40 days.
	Cases in which syphilis is acquired non-venerally [as occupationally in doctors] primary chancre is *extragenital usually on fingers.*
	Cases in which syphilis is transmitted by blood transfusion chancre don't occur.
	Persistent or multiple chancres may be seen in HIV infected or other immunodeficient patient.
Secondary syphilis	3 months after primary lesion.
	Reseolar or papular skin rashes, mucous patches in oropharynx and condylomata at mucocutaneous junction are characteristic lesions. Spirochetes are abundant in the lesions. These condylomata lata are highly infectious and are *most common* in groin or inner thigh.
	Patient *is most infectious* during this stage.
Tertiary syphilis	Consist of *cardiovascular lesions; chronic granuloma (gummata) & meningovascular manifestations such as tabes dorsalis.*

Remember: Latent syphilis: – Period of quiescence between secondary and tertiary stage.
– During this period diagnosis is only possible by serological test.

2. **Congenital syphilis**
   - *Woman with early syphilis is more infective* to her child.
   - Transmission across placenta can take place at any time, but lesions of congenital syphilis have their onset after 4th month of gestation. So, adequate treatment of mother before 4th month of pregnancy prevents fetal damage.
   - **Earliest sign** of congenital syphilis is rhinitis or snuffles. *....Harrison 19/e, p 1136*
   - ***MC*** early **manifestation** are bone changes, hepatosplenomegaly, lymphadenopathy.
   - ***Clutton's joint*** *(Bilateral knee effusion),* ***interstitial keratitis*** are late manifestation.
   - Residual stigmata of congenital syphilis are:

Hutchinson's teeth	:	Centrally notched, widely placed upper central incisor.
Mulberry molars	:	Sixth year molars with poorly developed cusps.
Rhagades	:	Linear scars at angle of mouth

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T-pallidum
- Microaerophilic
- Enters tissue by penetrating intact mucose or abraded skin
- Primary syphilis: Chancre
- Secondary syphilis: Macular or pustular lesion on the trunk and extremities
- Tertiary: Neurosyphilis, cardiovascular syphilis, gummatous syphilis.
- Lesions of secondary syphilis are most infectious.

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Woman with early syphilis is more infective to her child

Diagnosis

1. *Demonstration of Organism*
   - **Darkfield microscopic examination of lesion** exudate such as chancre of primary syphilis or more reliably by immunofluorescence or immunohistochemical method.
2. *Serological test for syphilis*

Nontreponemal test	Treponemal test
Detect IgG or IgM against cardiolipin antigen	Detect specific antibody against T. pallidum
Includes: *RPR* (Rapid plasma reagin) *[test of choice for rapid diagnosis]*	Includes: Fluoroscent treponemal antibody absorption test **[F-TAABS]**
VDRL [test of choice for response to therapy and for examining CSF	– Agglutination assays **(MHATP, TPHA, TPPA)**
VDRL is type of slide flocculation test while Kahn flocculation is tube test	– TPI (Treponemal pallidum immobilisation) test
Group specific treponenal tests: • These tests used the Reiter treponemes to avoid biological false positive • Reiter protein complement fixation (RPCF) test is the most commonly employed group specific treponemal test. • RPCF uses a lipopolysaccharide - protein complex antibody using a lipopolysaccharide - protein complex	

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FTA-ABS is the most sensitive test for syphilis TPI is the most specific test.

Remember:
- *FTA-ABS, TPPA are most sensitive test. FTA - ABS is (+) ve in approx. 80%, 100% & 95% of primary, secondary and late syphilis respectively* *[Greenwood 18/e p 370]*
- *TPI is most specific serological test.*
- *TPPA has supplanted the FTA-ABS test as diagnostic test.* *... CMDT 2014, p 1419*

3. **EIA and CIA (chemiluminescence)**
   - These test are simple sensitive specific and yield rapid result so they are now the initial test for screening. *...CMDT 2014, p 1419*
4. **Diagnosis of neurosyphilis**
   - Examination of CSF for pleocytosis, increase protein concentration, VDRL reactivity.
5. **Diagnosis of congenital syphilis**
   - If both test i.e. **VDRL** and **FTA- ABS IgM** (specific) test are positive in the infant then congenital syphilis should be strongly suspected and the child should be treated.

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Diagnosis of congenital syphilis: FTA-ABS IgM

Treatment

Stage of syphilis	DOC	Patient with penicillin allergy
Primary secondary or early latent	Penicillin benzathine	Tetracycline or Doxycycline
Late latent	Penicillin G	Tetracycline
Neurosyphilis	Aqueous penicillin G	Desensitization and treatment with penicillin
Syphilis in pregnancy	Penicillin	Desensitization and treatment with penicillin

Jarisch-Herxheimer reaction:
- May occur after penicillin therapy. It is characterized by fever, malaise and exacerbation of symptoms
- Seen in 50% of patients with primary syphilis, 90% of those with secondary disease and in a lower proportion with late stage disease
- It is thought to be a response to lipoproteins released from dying T. pallidum
- Subsides itself in all cases of primary and secondary syphilis. But it may be dangerous in some cases of gummatous cardiovascular and neurosyphilis
- ***Treatment:*** Symptomatic - Antibiotic should not be stopped. Steroids should not be given.

Evaluation of response to therapy:
- Response to treatment is determined by monitoring VDRL or RPR titer.
- Activity *of neurosyphilis is* best correlated with CSF pleocytosis.

Remember: Continued susceptibility of treponema to penicillin is due to its highly conserved genome.

NON-VENEREAL TREPONEMATOSIS

Infection usually transmitted by body to body contact. It includes:

A. Endemic syphilis: (Bejel)
- Caused by *T. pallidum* subspecies endemicum
- Disease is common in young children, primary chancre is not usually seen.
- ***Treatment:*** Same as venereal syphilis.

B. Yaws:
- Caused ***by T. pallidum*** subspecies ***T. pertenue*** which is morphologically and Immunologically identical to T. pallidum. *(Learn Py.)*
- Primary lesion is extragenital papule which enlarges and ulcerate to form an ulcerating granuloma.

C. Pinta:
- *Causative agent T.carateum (Learn Cap).*
- It is not identical but closely related to T. pallidum.
- ***Primary lesion*** is extragenital papule which doesn't ulcerate but develop into lichenoid or psoriatic patch.

Remember: In non-venereal treponematosis, yaws (***always***) and pinta (***usually***) ***serological test of syphilis are positive.***

LEPTOSPIRA

Actively motile spirochetes possessing a large number of closely wound spirals and characteristic ***hooked ends.***

Genus contains two species **L. interoorgans** being **pathogenic** and **L. biflexo** which is **saprophytic**

- **Culture:** Grow *best* under **aerobic condition at 28-32°C**. Leptospirae derive energy from oxidation of long chain fatty acid and cannot use aminoacid or carbohydrate as major energy source. So, for isolation **EMJH** media is used.
- **Fletcher medium** is another selectives media.

Pathogenesis and clinical manifestation:
- Leptospirosis is a **zoonosis** with ***rodents*** being most important reservoir.
- **Transmission** results from ingestion or contact with urine, blood or tissue from infected animal but not from bite. Since leptospires are excreted in urine of infected rat, *water is important vehicle.* Human to human transmission is rare.
- **Vasculitis is responsible for most clinical manifestation.**
- It mainly infects: - Liver *(centrilobular necrosis is found)*
  - Kidney *(cause interstitial nephritis, tubular necrosis)*
- After formation of antibody, leptospires are eliminated from all sites except *the eye, proximal renal tubules and brain.*
- More than 40% of symptomatic person have mild and anti-icteric form of leptospirosis.
- Severe leptospirosis is characterized by **profound jaundice, renal dysfunction, hemorrhagic diathesis called as** Weil's syndrome or Icterohemorrhagic fever.

Diagnosis

1. Direct demonstration	– PCR, dark field examination of centrifuged urine
2. Isolation of organism	– From blood or CSF during 1st 10 days
	– From urine after 1 week
	– For *isolation EMJH medium is useful*
3. Serology Type specific (indicates serovar)	– **Microscopic agglutination test [MAT] (Gold Standard)**
	– **Macroscopic agglutination test**
Gene specific (Identity infection without indicating serovar)	– **ELISA**
	– Sensitized erythrocyte lysis
	– CFT
	– Indirect immunofluorescence

Leptospira
- Zoonosis with widest geographical distribution.
- Rodents are the most important reservoir
- Mainly affects liver and kidney where it damages endothelial cells to produce vasculitis.

Compared with serology PCR offers a great advantage, particulary early diagnosis within first 5 days of illness

Treatment

- *Mild* - Doxycycline or Ampicillin.
- *Moderate/severe* - Penicillin or Erythromycin.

Chemoprophylaxis: Doxycycline

BORRELIA

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Borrelia
Causative agent of:
- **Relapsing fever:** Borrelia recurrentis
- **Lyme disease:** B. burgdorferi. The most common vector borne (Ixodid tick) infection in Europe.

- Large, refractile spirochetes which can be ***stained by ordinary method*** and are Gram (–) ve.
- Pathogenic species are:

B. burgdorferi	-	Causes Lyme's disease
B. recurrentis	-	Causes Relapsing fever
B. vincenti	-	Causes Vincent angina

LYMES' DISEASE (LYME BORRELIOSIS)

- Causative agent ***B.burgodorferi.***
- *MC* vector born infection in **Europe. Transmitted by bite of Ixodid ticks.**
  .... Harrison 19/e, p 1149, 18/e, p 1401
- **Clinical features**
  a. ***Stage I. (Localized infection)***
     - After incubation period of 3 to 32 days EM occurs at the site of bite. EM (Erythema migrans) is not painful.
  b. ***Stage II. Disseminated infection***
     - Disseminate hematogenously to produce secondary annular skin lesion, meningitis, carditis (*MC* cardiac finding is fluctuating degree of Atrioventricular heart block).
  c. ***Stage III. Persistent infection***
     - Usually present as oligoarticular arthritis (***MC*** knee), encephalopathy, polyneuropathy. Acrodermatitis chronica atrophicans is late skin manifestation.
- **Diagnosis**
  - ELISA followed by western blot is *best investigation.*
  - $VIsEC_6$ peptide IgG ELISA is the most promising second generation serological test.
  - Culture in BSK medium gives definitive diagnosis but not useful clinically.
  - PCR particularly in persistent infection.
- **Treatment**
  - For **nervous manifestation** and **3° heart block** – *Ceftriaxone* is *DOC.*
    ..... Harrison 19/e, p 1153, 18/e, p 1405
  - For **skin manifestation, arthritis 1° and 2° AV block** – *Doxycycline* is *DOC.*

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For diagnosis of Lyme's disease serological tests are preferred.
Harrison 19/e p 1151

RELAPSING FEVER

- Causative agent ***B. recurrentis.***
- It is of 2 types:
  - ***Louse borne*** and *Tick borne, Borrelia* causing them are indistinguishable.
  - ***Louse borne*** relapsing fever occur as ***epidemic.***
  - ***B. recurrentis*** is an *exclusive human pathogen* transmitted from person to person through body lice (Pediculus humanus corporis)
  - ***Tick borne*** occur as ***sporadic/endemic*** cases.
- **Treatment:** Erythromycin is *DOC.*

VINCENT ANGINA

- Causative agent ***B. vincenti***
- Normal mouth commensal, but when associated with fusiform bacilli (Fusobacterium fusiform) causes ulcerative gingivostomatitis or oropharyngitis called vincent angina.
- **Treatment:** Penicillin and Metronidazole.

Multiple Choice Questions

Syphilis

1. Congenital syphilis can be diagnosed by: [AI 01, 96]
a. IgM FTA BS
b. IgG FTA ABS
c. VDRL
d. TPI

2. False +ve VDRL is seen in: [AI 00, 95]
a. Lepromatous leprosy
b. Infectious mononucleosis
c. HIV
d. Pregnancy

3. False +ve VDRL is/are seen in: [PGI 2011]
a. Leprosy
b. Malaria
c. Relapsing fever
d. IV drug user
e. HIV infection

4. All are true about FTA-ABS Syphilis, except: [AI 00]
a. FTA-ABS becomes negative after treatment
b. Present in secondary syphilis
c. It is a sensitive test
d. May be positive in Lyme's disease

5. 'Chancre redux' is a clinical feature of: [AIIMS 06]
a. Early relapsing syphilis
b. Late syphilis
c. Chancroid
d. Recurrent herpes simplex infection

6. A 23-year-old male had unprotected sexual intercourse with a commercial sex worker. Two weeks later he developed a painless, indurated ulcer on the glans that exudated clear serum on pressure. Inguinal lymph nodes in both groins were enlarged and non-tender. Most approriate diagnostic test is: [AIIMS 04]
a. Gram's stain of ulcer discharge
b. Dark field microscopy of ulcer discharge
c. Giemsa stain of lymph node aspirate
d. ELISA for HIV infection

7. Best indicator for monitoring of syphilis treatment: [AIIMS 02]
a. VDRL
b. FTA-ABS
c. TPI
d. TPHA

8. Spirochaetes among following are: [PGI 06]
a. Syphilis
b. Leptospira
c. Mycoplasma
d. Brucella
e. Borrelia

9. True about primary chancre: [PGI Dec 2008]
a. Painless ulcer
b. Painless lymphadenopathy
c. Covered with exudate
d. Indurated lesion
e. Organism can be cultured from exudative fluid

10. False positive Nontreponemal serological test for syphilis are seen in: [PGI Dec 2008]
a. HIV Infection
b. Collagen disorders
c. Paediatric age group
d. Tuberculosis

11. Most sensitive test for Treponoma: [AIIMS 10]
a. VDRL
b. RPR
c. FTA-ABS
d. Kahn

12. A 25-year-old labourer presented 3 years back with penile ulcer which remain untreated. Later he presented with neurological symptoms for which he has taken appropriate treatment. Test to monitor response to treatment is: [AIIMS 09]
a. VDRL
b. TPI
c. FTA Abs
d. ELISA

13. A male patient presented with agitation, restlessness and neck stiffness. He had undergone treatment for penile ulcer - 3 years back. Lab investigation used for prognosis of treatment: [AIIMS 10]
a. TPI
b. VDRL
c. FTA-ABS
d. Dark field microscopy

Non-venereal Treponemas

14. Which is not true about Yaws? [AI 08]
a. Spread by sexual transmission
b. Caused by T. pertenue
c. Has cross immunity with syphilis
d. Cannot be differentiated serologically from T. pallidum

15. Non-venereal treponemas is/are: [PGI 04]
a. T. pertenue
b. T. carateum
c. T. palildum
d. T. cuniculi

16. About yaws all are true except: [AI 11]
a. Caused by T. pertenue
b. Transmitted non-venereally
c. Secondary yaw can involve bones
d. Later stages involves heart and nerves

17. True about Yaws: [PGI May 2013]
a. Sexually transmitted disease
b. Transmitted by fomites
c. Mother-child transmission
d. Periostitis occurs
e. Caused by T. pallidum subspecies endemicum

Leptospira

18. A bacterial disease with 3 'R's i.e. rats, rice fields and rainfall is: [AI 05]
a. Leptospirosis
b. Plague
c. Melioidosis
d. Rodent bite fever

19. A sweeper involved with repair-work of sewers was admitted with fever, jaundice and renal failure. The most appropriate test to diagnose infection of this patients: [AI 03]
a. Weill-Felix test
b. Paul-Bunnell test
c. Microscopic agglutination test
d. Microimmunofluorescence test

20. Which of the following is transmitted by Rat urine? [AI 02]
a. Leptospira
b. Listeria
c. Legionella
d. Mycoplasma

21. The following statements are true regarding leptospirosis, except: [AIIMS 06]
a. It is a zoonosis
b. Man is the dead end host
c. Man is an accidental host
d. Lice acts as reservoir of infection

22. 20-year-old boy has admitted with history of fever, icterus, conjunctival suffusion and hematuria for 20 days. Which of the following serological test can be of diagnostic utility: [AIIMS 04]
a. Widal test
b. Microscopic agglutination test
c. Paul-Bunnell-test
d. Weil-Felix reaction

23. Not used for diagnosis of Leptospirosis: [AIIMS 10]
a. Microscopic agglutination test
b. Dark field illumination
c. Macroscopic agglutination test
d. Weil-Felix reaction

24. True regarding leptospirosis is? [AI 11]
a. Rats are the only reservoirs
b. Fluoroquinolones are the DOC
c. Person to person transmission rare
d. Hepatorenal syndrome occurs in 50% cases

25. The following are true regarding Lyme's Disease, except: [AI 03]
a. It is transmitted by Ixodes tick
b. Erythema chronicum migraines may be a clinical feature
c. Borrelia recurrentis is the etiologic agent
d. Rodents act as natural hosts

Borrelia

26. A 25-year-old farmer presented with history of high grade fever for 7 days and altered sensorium for 2 day. On examination, he was comatosed and had conjunctival hemorrhage. Urgent investigations showed a hemoglobin of 11 gm/dl. Peripheral blood smear was negative for malarial parasite. What is the most likely diagnosis? [AIIMS 05]
a. Brucellosis
b. Weil's disease
c. Acute viral hepatitis
d. Q fever

27. Which one of the following microorganisms uses antigenic variation as a major means of invading host defenses: [AIIMS 04]
a. Streptococcus pneumonia
b. Borrelia recurrentis
c. Mycobacterium tuberculosis
d. Listeria monocytogenes

28. Lyme disease caused by: [PGI 01]
a. Leptospira
b. Borrelia
c. Treponema
d. Bordetella
e. Arbovirus

29. Which of the following species of borrelia cause relapsing fever: [AI 09]
a. Borrelia recurrentis
b. Borrelia hermsii
c. Borrelia turicatae
d. Borrelia duttonii

30. Tick born relapsing fever is/are caused by:
a. Borrelia recurrentis [PGI Dec 2008]
b. Borrelia duttonii
c. Borrelia burgdorferi
d. Borrelia hermsii
e. None of above

31. Lymes disease all are true except:
a. Borrelia burgdorferi replicates locally and invades locally [AIIMS May 10, May 11]
b. Infection progresses inspite of good humoral immunity
c. Polymorphonuclear leukocytoes in CSF suggest meningieal involvement
d. Intrathecal IgA confirms meningitis.

Explanations and References with Illustrative Answers

1. **Ans. (a) IgM FTAbs** *Ref. Ananthanarayan 9/e, p376; Harrison 16/e, p 984, 18/e, p 1384, 19/e, p 1136*

"Newborn infant of mother with reactive VDRL or FTA-ABS shows (+) ve test irrespective of infection because of trans-placental transfer of maternal IgG antibody."

As IgM antibody don't cross placenta, neonatal IgM antibody can be detected in cord or neonatal serum with the syphilis capita M or 195 IgM FTA-ABS test. — *Harrison 16/e, p 984*

Important points about Congenital syphilis:

- Transmission across placenta can take place any time, but lesion appear after 4 month of gestation.
- ***Earliest sign*** of congenital syphilis – Rhinitis, snuffles
- ***Residual stigmata*** of congenital syphilis – Hutchinson's teeth, Mulberry molars, Rhagades
- *DOC* of congenital syphilis – Penicillin G.

Adequate treatment of the woman before the 6th week of pregnancy should prevent fetal damage.

Among infants born alive only fulminant congenital syphilis is clinically apparent at birth.

Caution: According to *Harrison 18/e, p 1388* no commercially available IgM test is recommended for evaluation of infant with suspected congenital syphillis.

2. **Ans. (b) i.e. Infectious mononucleosis** *Ref. Harrison 18/e, p 1385, 19/e, p 1137; CMDT 2014, p 1419*

The modern VDRL and RPR test are 97% to 99% specific and false-positive test are now limited to following condition:

Causes of False-positive VDRL	
Acute false-positive reaction <6 months	**Chronic false-positive reaction > 6 month**
Recent viral illness or immunization	– Aging
Genital herpes	Autoimmune disorders
HIV infection	– SLE
Malaria	– Rheumatoid arthritis
Parenteral drug used	– Parenteral drug used
Other	
Infective endocarditis	– Hepatitis infection
Pregnancy	– Infectious mononucleosis

Infectious mononucleosis is acute infection of EBV and can give false (+)ve VDRL

- *In CMDT '08, p 1269* Leprosy is also mentioned as cause of false (+)ve VDRL: but with use of newer non-lipoidal VDRL test, leprosy no longer give false positive result.
- ***False negative*** **VDRL.** Seen when very high antibody titre is present **[Prozone phenomenon].**

Note: VDRL measures IgM and IgG directed against cardiolipin-lecithin-cholesterol antigen complex

3. **Ans. (b, c, d and e) i.e. Malaria, relapsing fever, IV drug user, HIV infection** *Ref. Harrison 18/e, p 1385, 19/e, p 1137-1138; CMDT 2014, p 1419*

Already explained

4. **Ans. (a) FTA-ABS becomes negative after treatment** *Ref. Harrison 17/e, p 1045, 18/e, p 1388*

- *Only* **VDRL** *and* **RPR** *for syphilis becomes negative after treatment and are recommended for evaluation of therapy. VDRL or RPR titer progressively declines, becomes (–ve) by 12 months in 40-75% of primary cases and in 20-40% of secondary cases.*
- **FTA-ABS** *and agglutination test remains positive after treatment, so these test are not useful in evaluating the response to therapy. HIV coinfected patients are less likely to become nonreactive.*

Follow-up Evaluation after Therapy for Syphilis		
Stage of Syphilis	Tests to Perform	When to Perform
Primary or Secondary	Quantitative RPR or VDRL[b]	HIV-uninfected: 6 and 12 months HIV-infected: 3, 6, 9, 12 and 24 months
Latent or late	Quantitative RPR or VDRL[b]	HIV-uninfected: 6, 12, 24 months HIV-infected: 6, 12, 18 and 24 months
Neurosyphilis (Asymptomatic or symptomatic)	1. If CSF pleocytosis was documented initially, repeat CSF exam. 2. Monitor decline in CSF protein and CSF-VDRL. (Note: Rate of decline may be slow). 3. Quantitative serum RPR or VDRL[b]	1. Every 6 months until CSF cell count is normal 2. Until normal 3. 6, 12, 18 and 24 months
[b]Try to distinguish between reinfection and treatment failure. If no clear evidence of reinfection exists, perform CSF examination. If CSF is normal, treat as for late latent syphilis.		

5. **Ans. is (a) i.e. Early relapsing syphillis** *Ref. Br J Clin Prac 1978:32 206-207*

Chancre redux or chancre monoredive is thought to be due to insufficient treatment of a syphilitic chancre, which after disappearance may relapse and accompanied by enlarged lymph nodes and the presence of numerous treponemes at the site of recurrence.

6. **Ans. (b) Dark field microscopy of ulcer discharge**
Ref. Ananthanarayan 8/e, p 373, 9/e, p 373; Harrison 16/e, p 1040, 18/e, p 1384, 19/e, p 1136-1138

Painless indurated ulcer (Hard chancre) with non-tender inguinal lymphadenopathy signifies diagnosis of syphilis.

Organism examine under dark ground microscope are:
- Leptospira - Treponema - Vibrio cholera - Campylobacter jejuni.

Mnemonic - **Local Train Via Chandigarh**

Diagnosis of Syphilis

a. Demonstration of organism:
- ***Dark field microscopic examination*** of lesion exudate is useful in moist cutaneous lesion such *as chancre* of primary syphilis; *condylomata* of secondary syphilis.
- A treponemal *concentration of* $>10^4$*/ml in* exudate is required for visibility under dark field microscope
  - *Direct fluorescent antibody T. pallidum (DFA-TP) test* – Use fluorescent conjugated antibody for detection of T. pallidum in fixed culture.

b. Serological test for Syphilis

Non-treponemal test	Treponemal test
– Detect antibody against cardiolipin	– Detect specific antibody against T. pallidum antigen
Includes	**Includes**
• VDRL (test of choice for response to therapy); RPR (test of choice for rapid diagnosis)	• FTA-ABS **(most sensitive test)**
• RPR (Rapid Plasma Reagin)	• Agglutination assays (MHA-TP; TPHA; TPPA)
• TRUS (Toluidine red unheated serum test)	• TPPA has supplanted the FTA-ABS test as diagnostic test.
	• TPI - **most specific serological test**. (not used now)

7. **Ans. (a) VDRL** *Ref. Harrison 17/e, p 1043, 18/e, p 1384, 19/e, p 1137*

Serological test of syphilis	
• *Most sensitive test*	FTA-ABS
• *Most specific test*	TPI (not used now)
• *Test of choice for rapid diagnosis*	RPR
• *Test of choice for evaluation of therapy*	VDRL
• *Test of choice for diagnosis of congenital syphilis*	IgM FTA-ABS
Now, TPPA has *supplanted* FTA-ABS as *definitive diagnosis test for syphilis.*	..... CMDT 2014, 1419

8. **Ans. (a), (b) and (e) Syphilis, *Leptospira* and *Borrelia*** *Ref. See below*

Spirochete	Species	Diseases
Treponema	T. pallidum T. endemicum T. pertenue	– Syphilis – Bejel/Endemic syphilis – Yaws
Borrelia	T. carateum B. burgdorferi B. recurrentis B. vincenti	– Pinta – Lyme disease – Relapsing fever – Vincent's angina
Leptospira	L. interrogans L. canicola	– Weil's disease – Canicola fever

9. **Ans (a,b,c,d) Painless ulcer, Painless lymphadenopathy, Covered with exudate, Indurated lesion** *Ref. Harrison 17/e, p 1040, 18/e, p 1382, 19/e, p 134; Textbook of Microbiology by DR Arora 3/e p 330, 331*

Primary chancre (Hunterian chancre)

- The primary lesion of syphilis is the chancre which usually begins as a single painless papule that rapidly become eroded and usually gets indurated.
- It is relatively avascular and has characteristic cartilaginous consistency.
- **Site:** It is located at the entry site of spirochete i.e. penis in heterosexual male, anal canal or rectum in homosexual; cervix and labia in females.
- The regional lymph node are swollen, discrete, rubbery and non-tender.
- Large number of treponemes are present in primary lesion and in the serum that exudes from it.
- *T. pallidum* do not grow in artificial media and so they cannot be cultured.

10. **Ans (a, b) HIV infection, Collagen disorders** *Ref. Harrison 16/e, p 984, 18/e, p 1384, 19/e, p 1137; Ananthanarayan 8/e, p 375, 9/e, p 375*

Non-treponemal test

Serological test in which cardiolipin or lipoidal antigen is used. Includes:

- Wasermann reaction – Kahn test
- VDRL – RPR

- As cardiolipin antigen is present in both *T. pallidum* and mammalian tissue, reagent antibody may be induced which accounts for biological false positive (BFP).

11. **Ans. (c) *FTA-ABS*** *Ref. Harisson 17/e, p 1045, 19/e, p 1137*

Most sensitive test for syphilis: FTA-ABS
Most specific test for syphilis: TPI

Note: Treponemal test are likely to remain reactive even after adequate treatment and cannot differentiate past from current treponemal infection.

12. **Ans. (a) VDRL** *Ref. Harrison 19/e 1135, 18/e, p 1385*

- **This is a case of neurosyphilis.** The efficiency of treatment of neurosyphilis is assessed by monitoring of quantitative VDRL or RPR Titer.
- FTA Abs agglutination test remain positive in most patients treated for seropositive syphilis, so these tests are not useful for monitoring the response.
- VDRL test can be performed on both serum and CSF, while RPR can not be performed on CSF, this makes VDRL test of choice for monitoring response particularly in neurosyphilis.
- CSF VDRL is highly specific and when reactive is considered diagnostic of neurosyphilis.

Remember: The activity of neurosyphilis is correlated best with CSF pleocytosis and this measure provides the most sensitive index of response to treatment.

13. **Ans (b) *VDRL*** *Ref. Harisson 19/e, p 1137, 18/e, p 1388*

Already explained.

14. Ans. (a) Spread by sexual transmission *Ref. Harrison 17/e, p 1047, 18/e, p 1390*

Yaws is caused by *T. pallidum* subspecies pertenue.

- *T. pertenue* is antigenically identical to T. Pallidum.

Mode of transmission : Infection is transmitted by direct contact with infectious lesions, often during play or group sleeping. Fly acts as mechanical vector.

Clinical features : After 3-4 weeks the initial lesion begins as a papule usually on extremity which enlarges and breaks down to form an ulcerating granuloma (mother yaw). It is followed by appearance of multiple skin lesion.

As in syphilis secondary and tertiary manifestations follows but cardiovascular or neurological involvement is rare. Destructive lesions of bones are common.

Treatment of choice : Benzathine penicillin.

15. Ans. (a) and (b) *T. pertenue* and *T. carateum* *Ref. Ananthanarayan 8/e, p 378, 9/e, p 377*

Non-venereal Treponematosis	
Endemic syphilis	– Caused by *T. pallidum* subspecies endemicum. – Transmitted by body to body contact. – Mainly seen in young children. – Primary chancre is not formed. – ***Treatment*** - penicillin is ***DOC***.
Yaws (=pian = Parangi)	– Caused by *T. pallidum* subspecies *T. pertenue*. – Primary lesion is extragenital papule which ulcerate to form an ulcerating granuloma.
Pinta	– Caused by *T. carateum* – Not identical but closely related to *T. pallidum* – Primary lesion is extragenital papule which does not ulcerate but develop into lichenoid or psoriatic patch.

Remember: *T. pallidum subspecies endemicum* and *T. pertenue are morphologically* and *immunologically identical* to *T. pallidum subspecies pallidum* (causative agent of syphilis). So VDRL is positive.

16. Ans. (d) i.e. Later stages involves heart and nerves *Ref. Harrison 17/e, p 1046, 18/e, p 1390*

Feature	Yaw (pian, framboesia)	Pinta (carate, azul)
Organism	T. pallidum subspecies pertenue	T. carateum
Mode of transmission	Skin to skin	Skin to skin
Usual age	Early childhood	Late childhood
Primary lesion	Ulcerative Papilloma (Mother yaw)	Non ulcerative papule with satellite
Site	Extremities	Extremities, face
Secondary lesion	Cutaneous papulosquamous	Pintides, pigmented, pruritic
	lesion, osteoperiostitis	
Relapses	Common	Rare
Late complication	Destructive gummas of skin, bone, cartilage	Nondestructive macules
Treatment	Benzathine penicillin	Benzathine penicillin

17. Ans. (b, d) Transmitted by formites, Periostitis occurs *Ref. Ananthanarayan 9/e, p 377; Harrison 18/e, p 390*

Painful papillomatous lesions on the soles of the feet, periostitis, polydactilitis are the secondary manifestations of yaws.

Late yaws also manifest as *gummas* of skin and long bones, hyperkeratosis of palm and soles, osteitis and periostitis and hydrarthrosis.

18. Ans. (a) Leptospirosis *Ref. Ananthanarayan 8/e, p 383, 9/e, p 383; Harrison 19/e, p 1141, 18/e, p 1393*

Leptospirosis

- ***Dead end infection*** of leptospira characterized by clinical manifestations ranging from inapparent infection to fulminant icterohemorrhagic fever ***(Weil's syndrome).***

Modes of transmission:

- **Rodents** are most important reservoir.
- Transmission occur by direct contact with urine, blood or tissue from an infected animal.

- Indirect contact: Contact of broken skin or ingestion of soil, water or vegetation contaminated by urine of infected animal.
- Direct man to man transmission is rare
- **Water is** an important vehicle of transmission.
- Out breaks mostly occur as a result of heavy rainfall and consequent flooding.

So, guys Leptospirosis is associated with: – Rats
– Rainfall
– Rice - Rats usually live in rice farms.

Other options

- **Heavy rainfall** tends *to protect against plague and Rodent bite fever* by damaging rodent burrows.
- Melioidosis is not associated with Rat.

19. **Ans. (c) Microscopic agglutination test** *Ref. Harrison 19e, p 1144, 18/e, p 1395; Ananthanarayan 8/e, p 384, 9/e, p 381*

- The patient is a case of ***Weil's syndrome*** or ***icterohemorrhagic fever*** which is caused by **Leptospira** and manifest as:
  - *Fever*
  - *Jaundice*
  - *Renal failure (Hematuria)*
- Human infections are usually due to occupational exposure to urine of infected animal e.g.,
  - Farmers
  - Workers in underground Sewers
  - Veterinarians
  - Workers in rice field and sugarcane field
  - Meat and animal handlers

Diagnosis	
Isolation of organism	**Serology**
EMJH medium is useful	Raise in antibody titre (>: 100) in *microscopic agglutination test (MAT).*
Dark field examination of patient blood	*Macroscopic agglutination test* is useful for screening but is *not specific.*
	IgM enzyme-linked immunosorbent assay (EIA) - particularly useful in making an *early* diagnosis.

Remember:
- Leptospirosis is most widespread zoonotic disease in world.
- Vasculitis is responsible for most manifestation of Leptospirosis
- Penicillin G is **DOC** for Leptospirosis.
- Fletcher medium and Korthof medium can also be used for isolation of leptospira.

20. **Ans. (a) *Leptospira*** *Ref. Ananthanarayan 8/e, p 383, 9/e, p 381; Harrison 19/e, p 1144, 18/e, p 1392*

Already explained

21. **Ans. (d) Lice acts as reservoir** *Ref. Park 22/e, p 266; Harrison 19/e, p 1142, 18/e, p 1393*

"Reservoir of leptospirosis are rats not lice." Other wild mammals as well as farm animals may also harbor leptospires.

- Most important source of infection are rats, dogs, cattle and pig.
- Human infection is ***mostly accidental.***
- *Transmission occur through direct contact Leptospira enter the body through:*
  - Skin abrasions or through intact mucous membrane.
  - Ingestion of food and water contaminated with urine of rat (minor important).
  - Inhalation of droplets of urine of infected animal.
- Direct man to man infection is rare.

22. **Ans. (b) Microscopic agglutination test** *Ref. Harrison 19/e, p 1144, 18/e, p 1395; Ananthanarayan 7/e, p 392, 8/e, p 383*

Already explained

23. **Ans (d) *Weil-Felix reaction*** *Ref. Ananthanarayan 8/e, p 384, 9/e, p 383*

Already explained

24. **Ans. (c) i.e. Person to person transmission rare** *Ref. Park 21/e p 267; Harrison 19/e, p 1142; 18/e, p 1397*

Already explained

Note:
- Weil syndrome develops in 5-10% of infected individual
- Treatment of choice for leptospirosis is Ampicillin
- Doxycycline is the drug of choice for chemoprophylaxis

25. Ans. (c) *Borrelia recurrentis* is the etiological agent

Ref. Ananthanarayan 8/e, p 381, 9/e, p 380; Harrison 19/e, p 1149, 18/e, p 1397

"Lyme's disease is caused by Borrelia burgdorferi not B. recurrentis."

Lyme's disease

- *Causative agent* – *Borrelia burgdorferi*
- *Vector* – Ixodes tick
- Natural reservoir host – Rodents, deer and other mammals.

Clinical Manifestation

- ***Stage 1*** – After I.P of 3-32 days
  - Erythema migrans occur at the site of tick bite.
  - ***MC* site** - Thigh, groin, axilla
- ***Stage 2*** – Disseminate hematogenously to produce: – *Secondary annular skin lesion*
  - *Meningitis*
  - *Carditis*
- ***Stage 3 (Persistent infection)*** – Intermittent attacks of oligoarticular arthritis (***MC*** - Knee).
  Acrodermatitis atrophicans – Late skin manifestation.

Diagnosis

- ***Serology*** – ELISA followed by western blot can't distinguish active and inactive infection.
- ***Isolation of organism*** – *B. burgdorferi* may be cultured from skin lesions of patient.
  - Grows best in BSK medium at 33°C.
- Detection of DNA by PCR, particularly in joint fluid.
- Later in infection PCR is greatly superior to culture.

Remember: *B. recurrentis* is etiological agent of Relapsing fever.

26. Ans. (b) Weil's disease *Ref. Harrison 19/e, p 1149, 18/e, p 1402*

This is **classic** case of **Weil disease** with: – Fever
- Jaundice
- Renal failure (increase urea).

27. Ans. (b) *Borrelia recurrentis* *Ref. Ananthanarayan 8/e, p 379, 9/e, p 378*

"Borrelia readily undergoes antigenic variations in vivo and this is believed to be the reason for relapsing in the disease." *.... Ananthanarayan 9/e, p 378*

Borrelia recurrentis

- Causative agent of epidemic or lice born relapsing fever

Remember: Other bacteria exhibiting antigenic variation are:
- Neisseria
- Group A streptococci

28. Ans. (b) *Borrelia* *Ref. Ananthanarayan 8/e, p 381, 9/e, p 380*

Already explained

29. Ans (a) *Borrelia recurrentis* *Ref. Ananthanarayan 8/e, p 379, 9/e, p 378*

Already explained

30. Ans (b) and (d) *Borrelia duttonii, Borrelia hermsii* *Ref. Ananthanarayan 7/e, p 386, 9/e, p 378*

Relapsing Fever	
Louse born (Epidemic)	Tick born (Endemic)
– *Borrelia recurrentis*	– *B. duttonii*
	– *B. hermsii*
	– *B. Parkeri*

31. Ans. is C i.e Polymorphonuclear leucocytosis in CSF suggest meningieal involvement

Spread of Infection in Lyme Disease *Ref. Ananthanarayan 8/e 381, 9/e, p 380; Harrison 19/e p 1150, 18/e, p 1401*

- In lyme disease B. burgdorferi in inoculated in skin by the bite of ixodes tick
- Bacteria replicates locally and migrate outward into the dermis.
- Days to week after the tick bite, hematogenous dissemination to secondary sites takes place. If untreated bacteria persist in the body for month or even years, despite the production of antibodies.
- **Following mechanism have been described for the resistance of B. burgdorferi:**
  - Tick saliva contains substance that disrupt immune response at the site of bite
  - Once inside the body, Borrelia changes shape. In the tick, bacterium has a thick cell wall and spiral shape. However, in the human body, Borrelia can shed its cell wall and can take a different shape. When under attack from immune system, it simply changes shape to remain unrecognized.
  - Borrelia burgdorferi produces antigenic variation in a "cell surface lipo protein VIsE", during the course of infection. by using this type of antigenic variation, B. burgdorferi can escape the adaptive immune system.

CSF Findings of Lyme Meningitis

- Pleocytosis with predominantly lymphocytosis (not polymorphonuclear leukocytosis)
- Increased protein
- Glucose content is usually normal, but it falls below the serum concentration in 20% of patients
- Immunoglobulin abnormalities are common in the CSF of patients with lyme meningitis.
  - Specific IgM, IgG or IgA antibody against B. Burgdorferi appears in CSF and indicates intrathecal antibody synthesis.

Chapter Review

1. Test used for diagnosis of congenital syphilis: [MP 05]
a. IgMFTA b. VDRL
c. TPI d. Kahn flocculation test
[Ref. Harrison 16/e, p 984]

2. The following is not true of syphilis: [AIIMS 91]
a. TPI is most specific
b. VDRL is not specific
c. VDRL is negative in secondary syphilis
d. IgM test is specific for congenital syphilis
[Ref. Harrison 19/e, p 1385]

"For Treponema pallidum immobilization test dark ground microscopy is required."
- **TPI** is *most specific* test for syphilis, but not performed now, as it requires Treponema in Tissue culture.
  - In TPI treponema is combined with antibody and complement of patient sample, if it results in immobilization (which is seen in dark ground, infection is confirmed).

[Ref. Ananthanarayan 8/e, p 376, 9/e, p 375]

3. Which is false about syphilis: [AI 92]
a. TPI gives definite diagnosis
b. TPHA is earliest to be +ve
c. VDRL is +ve 1 week after appearance of chancre
d. Serologically not different from yaws
[Ref. Jawetz 24/e, p 335]

4. Antigenic variations seen in: [MP 06]
a. *Borrelia recurrentis*
b. *Borrelia burgdorferi*
c. *Borrelia vincentii*
d. None *[Ref. Ananthanarayan 8/e, p 379, 9/e, p 378]*

5. The causative agent of Lyme's disease:
a. *Borrelia burgdorferi* [SGPGI 07; MP 05]
b. *Borrelia recurrentis*
c. *Leptospira icterohaemorrhagiae*
d. *Clostridium difficile*
[Ref. Ananthanarayana 8/e, p 381, 9/e, p 380]

6. Consider the following statements:
1. In leptospirosis, the incubation period ranges from 2 to 20 days
2. In leptospirosis, urine may show microscopic haematuria

Which of the following statement are correct:
a. 1 only b. 2 only [UPSC 07]
c. Both 1 and 2 d. Neither 1 nor 2
[Ref. Harrison 18/e, 1393,1394]

7. Which of the following investigation is very specific for spirochaetal infection? [Kar 03]
a. Wassermann's test
b. VDRL test
c. Treponema pallidum
d. Fluorescent treponemal antibody absorption test
[Ref. Ananthanarayan 8/e, p 376, 9/e, p 375]

8. Organisms that has not been cultured successfully so far is: [J & K 01]
a. Leptospira b. Treponema pallidum
c. Bordetella d. Staphylococcus
[Ref. Ananthanarayan 8/e, p 372, 9/e, p 371]

9. Yaws is caused by: [Delhi 08]
a. *Treponema pallidum*
b. *Campylobacter jejuni*
c. *Salmonella*
d. *S. aureus* *[Ananthanarayana 8/e, p 376, 9/e, p 377]*

10. Genetic variations are important in: [Delhi 07]
a. *Borrelia* b. *S. aureus*
c. *Leptospira* d. *Haemophilus*
[Ananthanarayana 8/e, p 379, 9/e, p 378]

11. Which of the following disease is not caused by treponema: [Kar 00]
a. Yaws b. Bejel
c. Relapsing fever d. Syphilis
[Ref. Ananthanarayana 9/e, p 378]

12. *Treponema pallidum* is very difficult to demonstrate in: [JIPMER 02]
a. Chancre
b. Maculopapular lesion
c. Gumma
d. Mucosal patch *[Ref. Harrison 17/e, p 1042]*

13. The most commonly performed test for the serodiagnosis of syphilis is: [Kar 2002]
a. TPHA b. Wassermann
c. TPI d. VDRL
[Ref. Ananthanarayan 8/e, p 375, 7/e, p 381]

Answers 1. a IgMFTA 2. c. VDRL ... 3. b. TPHA is earliest ... 4. a. *Borrelia* ... 5. a. Borrelia ...
6. c. both ... 7. d. Fluorescent 8. b. *Treponema* ... 9. a. *Treponema* ... 10. a. Borrelia
11. c. Relapsing ... 12. c. Gumma 13. d. VDRL

14. Syphilis was first identified by: [TN 02, DNB 2013]
a. Fraenkel
b. Nicolaescu
c. Schaudinn and Hoffman
d. Ogston *[Ref. Ananthanarayana 8/e, p 372]*

15. Syphilis is diagnosed by all except: [UP 02]
a. TPI
b. FTA-ABS
c. Weil-Felix
d. VDRL *[Ref. Ananthanarayana 9/e, p 374]*

16. Consider the following serological tests:
1. FTA-ABS 2. TPI
3. TPHA 4. VDRL

Which of these are specific for diagnosing syphilis?
a. 1 only b. 2 and 4 [Bihar 03]
c. 3 and 4 d. 1, 2 and 3
[Ref. Ananthanarayan 8/e, p 375, 9/e, p 374]

17. Which of the following is the most specific test to diagnose syphilis: [Comed 07]
a. VDRL test
b. Wassermann test
c. RPR
d. FTA-ABS *[Ref. Ananthanarayan 7/e 384, 9/e 375]*

18. Most specific test for syphilis: [Kolkata 03]
a. FTA-ABS b. TPI
c. VDRL d. ELISA
[Ref. Ananthanarayan 8/e, p 376, 9/e, p 375]

19. Nichol's treponemes are used in: [Delhi 06]
a. Wassermann reaction
b. VDRL
c. RPR test
d. TPI test *[Ananthanarayana 8/e, p 372, 9/e, p 372]*

Nichol's strain was isolated from the brain of fatal case of neurosyphilis in 1912 and is still being propagated by serial testicular passage in rabbit. It is used for research purpose and diagnostic purpose in treponema specific tests.

20. Dark ground microscopy is used for: [PGI 98]
a. TPI
b. FTA-ABS
c. Kahn's test
d. VDRL
[Ref. Ananthanarayan 8/e, p 376, 9/e, p 375]

- **TPI** is *most specific* test for syphilis, but not performed now, as it requires Treponoma in Tissue culture.
  - In TPI treponema is combined with antibody and complement of patient sample, if it results in immobilization (which is seen in dark ground, infection is confirmed).

21. Following is true of *T. pallidum*, except: [PGI 98]
a. Can be maintained in rabbit testis
b. Motile by peritrichate flagella
c. To visualise, dark ground microscopy is used
d. TPI test is very useful
[Ref. Ananthanarayan 8/e, p 372, 9/e, p 371]

22. RPR is done for diagnosis of: [DNB 2013]
a. Malaria
b. Syphilis
c. Leishmaniasis
d. None *[Ref. Ananthanarayan 9/e, p 374]*

23. Diagnosis of congenital syphilis is done by: [DNB 2013]
a. Culture
b. FTA-ABS
c. RPR
d. Not recalled
[Ref. Ananthanarayan and Paniker 7/e, p 39]

Answers **14.** c. Schaudinn and Hoffman **15.** c. Weil-Felix **16.** d. 1, 2 and 3 **17.** d. FTA-ABS
18. b. TPI **19.** d. TPI test **20.** a. TPI **21.** b. Motile by... **22.** b. Syphilis
23. b. FAT-ABS

NEET Pattern Questions

1. **Incubation period of syphilis:**
   a. 1 hour - 5 hour
   b. 24 hour - 48 hour
   c. 1 day - 10 days
   d. 10 days - 90 days *[Ref. Ananthanarayan, 9/e, p 372]*

2. **Stain for treponema:**
   a. Fontana's
   b. Acid-facid
   c. Methenamine-silver
   d. PAS *[Ref. Ananthanarayan, 9/e, p 371]*

> Treponema Pallidum is stained by silver impregnation methods. Fontana's method is useful for staining films and Levaditi's method for tissue section

3. **Borrelia undergoes antigenic variation due to:**
   a. Plasmids
   b. Transposons
   c. Intrinsic mutation
   d. All of the above *[Ref. Ananthanarayan, 9/e, p 378]*

> Antigenic variation of Borelia are due to DNA rearrangements in linear plasmids.

4. **Yaws is caused by:**
   a. Treponema pallidum
   b. Treponema carateum
   c. Treponema refringens
   d. Treponema pertenue *[Ref. Ananthanarayan, 9/e, p377]*

5. **Louse borne relapsing fever:**
   a. B duttoni
   b. B recurrentis
   c. B parkeri
   d. B burgdorferi *[Ref. Ananthanarayan, 9/e, p378]*

Relapsing fever	
Louse borne (epidemic)	Tick borne (Endemic)
B. recurrentis	B. duttoni B. hermsis B. parkeri

6. **Leptospirosis is transmitted by:**
   a. Rat
   b. Cat
   c. Dog
   d. Fish
   [Ref. Ananthanarayan, 9/e, p 383]

7. **Painless genital ulcer in male with everted margin is seen in:**
   a. Syphilis
   b. Chancroid
   c. Herpes
   d. LGV
   [Ref. Harrison, 18/e, p 1382]

8. **Dark field microscopy used in:**
   a. Vibrio
   b. Syphilis
   c. TB
   d. Brucellosis
   [Ref. Ananthanarayan, 9/e, p 371]

9. **Reservoir of leptospira:**
   a. Cat
   b. Dog
   c. Rat
   d. Monkey
   [Ref. Ananthanarayan, 9/e, p 383]

10. **Stain for Treponema:**
    a. Fontana's
    b. Acid-facid
    c. Methenamine-silver
    d. PAS *[Ref. Ananthanarayan, 9/e]*

> T. pallidum does not take ordinary bacterial stains but stains a light rose red with prolonged Giemsa staining. It can be stained by silver impregnation methods. Fontana's method is useful for staining films and Levaditi's method for tissue section.

11. **In a syphilis patient, site which does not help in isolation of organism:**
    a. Gumma
    b. Pr chancre
    c. Mucosal patch
    d. Maculopapular rash

12. **Confirmatory test for syphilis:**
    a. VDRL
    b. FTA-ABS
    c. RPO
    d. None *[Ref. Ananthanarayan, 9/e, p 378]*

13. **True about B. recurrentis:**
    a. Causes leptospirosis
    b. Water borne disease
    c. Vector borne disease
    d. Transmitted by tick *[Ref. Ananthanarayan, 9/e, p 378]*

Answers
1. **d.** 10 days - 90 days
2. **a.** Fontana's
3. **a.** Plasmids
4. **d.** Treponema pertenue
5. **b.** B recurrentis
6. **a.** Rat
7. **a.** Syphilis
8. **c.** TB
9. **c.** Rat
10. **c.** Methenamine-silver
11. **a.** Gumma
12. **b.** FTA-ABS
13. **c.** Vector borne disease

14. Tabes dorsalis is manifestation of:
a. Primary syphilis
b. Secondary syphilis
c. Tertiary syphilis
d. Latent syphilis
[Ref. Ananthanarayan, 9/e, p373; Harrison, 8/e, p 1383]

Tabes dorsalis is a late manifestation of syphilis that presents with symptoms and signs of demyelination of the posterior columns, dorsal root and dorsal root ganglia.

15. Syphilis was first identified b:
a. Fraenkel
b. Nicolaricu
c. Schaudinn and Hoffman
d. Ogston

16. Dark field microscopy used in:
a. Vibrio
b. Syphilis
c. TB
d. Brucellosis
[Ref. Ananthanarayan, 9/e, p 371]

17. Painless ulcer along with painless lymphadenopathy is characteristic of which STD:
a. Syphilis
b. Chancroid
c. LGV
d. Donovanosis
[Ref. Harrison, 19/e, p 880]

18. Vincent's angina is caused by Borrelia vincentii along with:
a. Lactobacillus
b. Peptostreptococcus
c. Fusobacterium
d. Bacteroides
[Ref. Ananthanarayan, 9/e, p 379]

19. Culture medium used for leptospira for laboratory diagnosis:
a. Skirrows medium
b. EMJH medium
c. BYCE agar
d. Pike's medium
[Ref. Ananthanarayan, 9/e, p 382]

20. Fletcher's medium containing Rabbit serum is used for:
a. Streptococcus
b. Bacillus anthracis
c. Leptospira
d. Borrelia

21. Most common form of leptospirosis:
a. Weil's disease
b. Icteric form
c. Hepatorenal form
d. Anicteric form
[Ref. Harrison, 19/e, p 1144]

Answers **14.** c. Tertiary syphilis **15.** c. Schaudinn... **16.** b. Syphilis **17.** a. Syphilis
18. c. Fusobacterium **19.** b. EMJH medium **20.** c. Leptospira **21.** d. Anicteric form

CHAPTER

23

Mycoplasma

- ***Smallest free living bacteria, that lacks cell wall and is*** bounded by sterols containing soft trilaminar membrane, so are highly pleomorphic.
- **Lack cell wall** so are resistant to cell wall active antimicrobial agent such as penicillin, cephalosporin and lysozyme.
- Also called as ***PPLO = Stable L forms.***
- Even cell precursors like *muramic acid or diaminopimelic acid are absent.*
- Do not possess spores flagella or fimbria. Some species exhibit ***gliding motility.***

Smallest free living bacteria
- Smallest organism capable of independent replication
- Lacks cells wall
- Exhibit biphasic colony with fried egg appearance
- Grows best at 35 to 37°C
- Causative agent of walking pneumonia.
- Smallest organism capable of independent replication.

Morphology

- Gram negative but better stained *by Giemsa.*
- Can be grown on cell free media, that contain lipoprotein and sterol.
- Colony is typically biphasic with **"Fried egg appearance" best studied** after staining *by Dienes* method. Most mycoplasma colonies are hemolytic.
- Pathogenic mycoplasma grows best at at 35-37°C. Media are enriched with 20% horse or human serum and yeast extract. Penicillin and thallium acetate are added as selective agents.
- Some species shows bulbous enlargement with a differentiated tip structure which aids in attachment of organism to host cell carrying neuraminic acid receptor.

Mechanism of Pathogenicity

- Adherence to host cell
- H_2O_2 production (as in *M. pneumonia*)
- Ammonia production (as in *M. hominis*).
- Urease activity to produce ammonia (as in *U. urealyticum*).
- IgM autoantibodies that agglutinate human group O erythrocyte at 4°C. This *cold agglutinin* produces anemia.

Classification

- *M. pneumoniae* – Upper and lower respiratory tract infection.
- *M. genitalium* and *Ureaplasma urealyticum* – Urethritis and other genital condition.
- *M. hominis* and *U. urealyticum* – *Part of flora of bacterial vaginosis.*

MYCOPLASMA PNEUMONIA = PRIMARY ATYPICAL PNEUMONIA = WALKING PNEUMONIA

- Cause by *M. pneumonia* (= Eaton agent) in which pneumonia is classic presentation but **non-pneumonic** infection is more common with prolonged incubation period.
- Interstitial type of pneumonia characterized by paucity of respiratory signs on auscultation with striking radiological abnormality.
- **Extrapulmonary manifestations** (e.g. Erythema multiforme, anemia, pancreatitis, coagulopathies) is due to autoantibodies against brain, heart and muscle.

Diagnosis

i. *Isolation*: Throat swab/respiratory secretions are inoculated into medium containing glucose and phenol.

ii. *Serological diagnosis:*
   a. **Specific test** - Immunofluorescence hemagglutination inhibition and metabolic inhibition are most sensitive test.
   b. **Non-specific serological test** are streptococcus MG (group F) and cold agglutination test.

I

- Non-gonococcal Urethritis: U. urealyticum and M. genitalium are the most common non-chlamydial cause of NGU.
- Cell wall deficient form of Gr(+) bacteria: Protoplast
- Cell wall deficient form of Gr(–) bacteria: Spheroplast.

GENITAL MYCOPLASMAS

- Non-gonococcal Urethritis (NGU) - *U. urealyticum* and *M. genitalium* cause most of non-chlamydial cases of NGU.
- *U. urealyticum* called as **T. strain/T. form of mycoplasma** use urea as a source of energy. Hence, ***Urea and cholesterol are essential growth factor.***

Treatment: Tetracycline [Doxycycline] is DOC for treatment of mycoplasma infection.

CELL WALL DEFECTIVE BACTERIA

L phase variants (L. forms)

- Wall defective microbial forms that can replicate serially as non-rigid cells and produce colonies on solid media.
- *Protoplasts* are such forms usually derived from Gram +ve organisms. They are osmotically fragile.
- *Spheroplasts* are cell wall defective form usually derived from Gram –ve bacteria. They retain outer membrane.
- These L forms results from spontaneous mutation or by the effect of chemicals.
- ***Reversion of L-form to the parental bacterial form is enhanced by growth in the presence of 15-30% gelatin or 2-5% agar.***

Multiple Choice Questions

1. **Atypical pneumonia can be caused by the following microbial agents except:** [AI 05]
   a. *Mycoplasma*
   b. *Legionella pneumophila*
   c. Human corona virus
   d. *Klebsiella pneumoniae*

2. **All are features of *ureaplasma urealyticum* except:** [AI 01]
   a. Non-gongococcal urethritis
   b. Salpingitis
   c. Epididymitis
   d. Bacterial vaginosis

3. **The following statements are true with references to Mycoplasma except :** [AIIMS 05]
   a. They are the smallest prokaryotic organisms that can grow in cell free culture media
   b. They are obligate intracellular organisms
   c. They lack a cell wall
   d. They are resistant to Beta-lactam drugs

4. **In reference to Mycoplasma, the following are true except:** [AIIMS 05]
   a. They are inhibited by penicillins
   b. They can reproduce in cell free media
   c. They have an affinity for mammalian cell membranes
   d. They can pass through fiters of 450 nm pore size

5. **The following is true about *Mycoplasma* except:** [AIIMS 02, 96]
   a. Multiply by binary fission
   b. Are sensitive to beta-lactam group of drugs
   c. Can grow in cell free media
   d. Requires sterols for their growth

6. ***Mycoplasma pneumonia* is characterized by all except :** [AIIMS 01]
   a. Diagnosed by serum cold antibody
   b. Treatment is erythromycin
   c. Cannot be cultured from sputum
   d. Raised ESR

Explanations and References with Illustrative Answers

1. **Ans. (d) *Klebsiella pneumoniae*** *[Ref. Robbin's 7/e, p 751]*

Atypical pneumonia is characterized by patchy inflammatory changes in the lung, largely confined to alveolar septa and pulmonary interstitium.

Causes of atypical pneumonia	
Mycoplasma (MC)	Coxiella burnettii (Q fever)
Legionella pneumonia	Pneumocystis carinii
Francisella tularensis	Histoplasma capsulatum
Chlamydia psittacosis, Chlamydia pneumoniae	Coccidiodis immitis.
Viruses (influenzae A and B, RSV, Adeno, rhino, rubeola, varicella, etc).	

Klebsiella is associated with community acquired pneumonia classically in alcoholics (also in diabetics and chronic lung disease). It usually affects upper lobes producing expansion of lobes (bulging fissure) and Red current jelly sputum.

Note: Causes of community acquired pneumoniae in decreasing frequency ***Strep. pneumoniae > H. influenza > Chlamydia > Legionella***.

2. **Ans. (b) Salpingitis** *[Ref. Harrison 19/e, p 1163; Ananthanarayan 8/e, p 389, 9/e, p 389; CGDT 9/e, p 654]*

***Ureaplasma urealyticum* are *T. form mycoplasmas* which are urease positive.**

It causes:

- Nongonococcal urethritis (*MC* cause is *Chlamydia trachomatis* also caused by *U. urealyticum* and *M. genitalium.*)
- Epididymitis **(no role of M. homins).**
- Chorioamnionitis
- Postpartum fever
- Proctitis
- Reiter's syndrome
- Acute salpingitis
- Pneumonia and chronic lung disease in very low birth weight infants
- PID and bacterial vaginosis : by *M. hominis* and *U. urealyticum*
- Infertility in both men and women
- Late abortion
- Low birth weight infant
- Balanoposthitis
- Cervicitis and vaginitis

... Not given in Harrison and CGDT

Bacterial vaginosis (Altered vaginal normal microbial flora) is associated with *Gardenrella* and *Hemophilus vaginitis*, in which **clue cells** are present and **Amide test** is positive.

If 'none' is given as option then it is more appropriate than 'Salpingitis' as salpingitis is given in few books.

3. **Ans. (b) They are obligate intracellular organisms** *[Ref. See below]*

- *Mycoplasma* is not obligate intracellular bacteria.
- ***Obligate intracellular bacteria :***
  - *M. leprae*
  - Chlamydia
  - Rickettsiae and Coxiella burnettii
  - Pathogenic treponemes.
- Obligate intracellular bacteria cannot grow in cell free media.

4. **Ans. (a) They are inhibited by penicillins** *[Ref. Ananthanarayan 8/e, p 387-89, 9/e, p 387]*

- Mycoplasmas are **devoid of cell walls** (but bound by soft trilaminar unit membrane of sterols) and so **they are resistant to** β. lactams (penicillin, cephalosporins, vancomycin, bacitracin) and lysozymes that act on cell wall.
- Due to lack of cell wall they are highly pleomorphic and pass through bacterial filters of 450 nm since size varies from 50-300 nm in diameter.
- Parasitic mycoplasma requires cholesterol or other sterols as an essential growth factor.
- They have affinity for mammalian cell membrane. *.....Jawertz 27/e, p 335*

- It typically colonizes mucosal surfaces of respiratory, gastrointestinal and genitourinary tracts.
- Mycoplasma occur as granules and filaments (shows *true branching*).
- They multiply by asynchronous binary fission producing budding forms and chains of beads.
- Some species get attached to suitable host cells carrying neuraminic acid receptors by bulbous enlargement.
- *Mycoplasmas can grow in cell free media.*
- **Media** of *Mycoplasma* are **enriched with 20% horse** or **human serum** and **yeast extract**
  - Penicillin and thallium are ***selective agents***.
  - Colonies is typically biphasic with a *fried egg appearance* and are best studied after staining by Dienes method.
  - Growth of mycoplasma is inhibited by specific antibody.

5. **Ans. (b) Are sensitive to beta-lactam group of drugs** *Ref. Ananthanarayan 8/e, p 387-389, 9/e, p 386 - 388*

Note: Doxycycline is drug of choice for mycoplasma.

6. **Ans. (c) Cannot be cultured from sputum** *Ref. Ananthanarayan 8/e, p 389, 9/e, p 387; Harrison 19/e, p 1163, 18/e, p 1418*
   - *Mycoplasma pneumoniae* is ***MC*** cause of Atypical pneumonia which is characterized by reticulonodular or interstitial infiltration of lower lobes on X-ray with paucity of signs on auscultation.
   - Diagnosis is mainly clinical. Lab test are of secondary value.

Lab Diagnosis

- ***Specimen:*** – Throat swabs, sputum or respiratory secretions.
- ***Microscopy:*** – It cannot be detected on Gram's stain as it lacks cell wall.
- – Gram's stain of sputum shows leukocytes without predominance of any bacteria morphologic type.
- ***Culture:*** It can be grown on artificial media but process is difficult as it requires special media and takes more than 2weeks so cultures do not provide timely information.
- ***Serology:***
  a. *Specific* : Antibodies are detected by enzyme linked immunoassays, indirect immunofluorescence, or complement fixation test.
  b. *Nonspecific* : Cold agglutinin aids in diagnosis since they develops within 7-10 days of infection and can be easily detected.
     - They are IgM autoantibodies which agglutinate human erythrocytes at 4°C,
     - Cold agglutinin titer > 1:32 supports diagnosis of *M. pneumoniae*.
     - It can also be performed at the bedside.
- ***Antigen Detection test:*** Includes antigen capture, indirect enzyme immunoassays, DNA probing and multiplex nucleic acid amplification test.

Note: EIA which detect IgG and IgM antibody is highly sensitive and specific.

Treatment

Ambulatory patients with community acquired pneumonia	Hospitalized patients with community acquired pneumonia
Oral doxycycline Oral erythromycin Oral clarithromycin, azithromycin, Levofloxacin, Moxifloxacin, ciprofloxacin	IV ceftriaxone or IV cefotaxime

Remember: Media for cultivating mycoplasma are enriched with 20% horse or human serum and yeast extract. Penicillin and thallium acetate are added as selective agent.

Chapter Review

1. **True about *Mycoplasma*:** **[UP 97]**
   a. CO_2 is important for growth
   b. Does not cause hemolysis
   c. May be commensal in throat
   d. Acid fast bacillus
   [Ref. Ananthanarayan 8/e, p 589, 9/e, p 622]

2. **True about *Mcyoplasma* is:** **[MP 98]**
   a. Not sensitive to antibiotics
   b. L. form is commonest
   c. Culture in sarbaroudz media
   d. Most common cause of nongonococcal urethritis
   [Ref. Ananthanarayan 8/e, p 390, 9/e, p 389]

3. **Positive cold agglutination test is seen in infections with:**
   a. *Mycoplasma* **[Manipal 08]**
   b. *Chlamydia*
   c. Infectious mononucleosis
   d. *Varicella* *[Ref. Ananthanarayan 8/e, p 389, 9/e, p 389]*

4. **True about *Mycoplasma* is:** **[AIIMS 95]**
   a. Causes lung infection
   b. Penicillin is drug of choice
   c. Thick cell wall
   d. Thallium acetate inhibits the growth
   [Ref. Ananthanarayan 8/e 387, 9/e 388]

5. **Cell wall deficient forms is:** **[DNB 2013]**
   a. Streptococcal
   b. Myoplasma
   c. Diphtheria
   d. None *[Ref. Ananthanarayan 9/e 389]*

Answers 1. **c.** May be ... 2. **b.** L. form is ... 3. **a.** Mycoplasma 4. **a.** Causes lung... 5. **b.** Myoplasma

NEET Pattern Questions

1. **Diene's method is used for:**
   a. Mycoplasma
   b. Chlamydiae
   c. Plague
   d. Diphtheria
   [Ref. Ananthanarayan, 9/e, p387]

   In Dienes method a block of afar containing the colony is cut and placed on a slide. It is covered with a cover slip on which on alcohol solution of methylena blue and azure has been dried.

2. **Ureaplasma is naturally resistant to:**
   a. Erythromycin b. Tetracycline
   c. Chloramphenicol d. Cephalosporins
   [Ref. Ananthanarayan, 9/e, p387]

3. **True about mycoplasma:**
   a. L-form b. Multidrug resistant
   c. Cause NGU d. Penicillin effective
   [Ref. Ananthanarayan, 9/e, p387, 389]

4. **True about mycoplasma:**
   a. Obligate intracellular organism
   b. Penicillin is effective treatment
   c. Require cholesterol for growth
   d. Have thick cell wall
   [Ref. Ananthanarayan, 9/e, p386]

   Media for cultivating mycoplasma are enriched with 20% horse or human serum and yeast extract. The high concentration of serum is necessary as a source of cholesterol and other lipids

Answers 1. **a.** Mycoplasma 2. **d.** Cephalosporins 3. **a.** L-form, **c.** Cause NGU 4. **c.** Require cholesterol for growth

Section - B

UNIT – II Virology

- DNA Virus
- RNA Virus
- Slow Virus Disease
- Hepatitis Virus
- HIV and Other Retrovirus

CHAPTER

24

DNA Virus

HERPES VIRUSES

- *Enveloped* virus with linear double stranded genome.
- Characterised by their ability to establish life long persistent infection in their host and to undergo periodic reactivation.
- Replicate in host cell ***nucleus*** forming **cowdry type A intranuclear** *(Lipshutz)* inclusion bodies.
- Virus are relatively thermolabile and readily inactivated by lipid solvents.

I

Herpes Virus
- Enveloped virus
- Forms cowdry type A intra-nuclear inclusion bodies

Classification of human herpes viruses

Species				
Official name	Common name	Sub-family	Cytopathology	Site of latent infection
Human herpesvirus type 1	Herpes simplex virus type 1	Alpha	Cytolytic	Neurons/gasserian or Trigeminal ganglia
Human herpesvirus type 2	Herpes simplex virus type 2	Alpha	Cytolytic	Neurons/sacral ganglia
Human herpesvirus type 3	Varicella zoster virus	alpha	Cytolytic	Neurons/T3 - L3 **(MC)**.
Human herpesvirus type 4	Epstein–Barr virus	Gamma	Lymphoproliferative	Lymphoid tissue (B cells)
Human herpesvirus type 5	Cytomegalovirus	Beta	Cytomegalic	Secretory glands Kidneys, (salivary glands and bowel)
Human herpesvirus type 6	Human B cell lymphotropic virus	Beta	Lymphoproliferative	Lymphoid tissues
Human herpesvirus type 7	R K virus	Beta	Lymphoproliferative	Lymphoid tissues
Human herpesvirus type 8		Gamma		

I
- Neurotrophic herpes virus: HSV and VZV
- Lymphotropic herpes virus: EBV, HHV-6 &7

- **HHV - 6** cause exanthem subitum/roseola infantum or ***sixth disease***.
- Different Herpes virus species don't show any antigenic cross reaction except Herpes simplex types 1 and 2.
- HHV-8 is associated with AIDS/Non-AIDS **Kaposi sarcoma.**

I

Site of latency:
1. HSV-1: Trigeminal ganglia, vagus ganglia, adrenal tissue and brain
2. HSV-1: Sacral ganglia

... *Greenwood, 18/e, p422*

HERPES SIMPLEX VIRUS (HSV)

- HSV includes HSV-1 and HSV-2, both of them are associated with variety of infections affecting both immunocompromised and immunocompetant individuals.

HSV Type 1	HSV Type 2
• Cause lesion in and around mouth	Cause lesion around genital area
• Transmitted by direct contact or droplet spread	Usually transmitted sexually
• Replicate poorly in chick embryofibroblast cell	Replicate well
• Relatlvely sensitive to antiviral agents	Resistant
• Less neurovirulent	More neurovirulent
• Infectivity is less temperature sensitive	More temperature sensitive
• Site of latency-trigeminal ganglia	Sacral ganglia
• On chick embryo CAM, form smaller pock	Form larger pock

I

Herpes simplex virus

Type I Fragile, less neurovirulent, cause lesion around mouth.

Type II Resistant, neurovirulent cause lesion around genital area.

- Genital herpes most frequently involves cervix and urethra in females and penis in males.
- In viscera oesophagus is the MC site.
- HSV-1 is the most common cause of sporadic encephalitis.
- HSV is the MC cause of Mollaret's meningitis.

- Genomes of HSV-1 and HSV-2 are similar and both cross react serologically. They can be distinguished by sequence analysis or by restriction enzyme analysis of viral DNA .

...Jawetz 27/e, p 460

Pathogenesis

- Humans are only natural host.
- ***Source of infection*** Saliva, skin lesion or respiratory secretion.
- On exposure mucosal surfaces or abraded skin permits entry of virus and initiates its replication in epidermis and dermis.
- On entry into neuronal cells the virus is transported intra-axonally (centripetally) to nerve cell bodies in ganglia.
- During initial phase of infection virus replication occur in ganglia, virus then spread to other mucosal surfaces through centrifugal migration of infectious virions via peripheral sensory nerves.
- Both antibody mediated and cell mediated immunity are important.
- ***CD8 + T cell*** responses are critical for clearance of virus from lesion.
- Typical lesion produced by HSV is the vesicle, a ballooning degeneration of intra-epithelial cells.

Clinical Features

- **Orofacial Infection**
  - Gingivitis and pharyngitis are most frequent clinical manifestation of first episode of HSV-1 infection **(primary infection)**.
  - Recurrent herpes ***labialis*** is ***MC*** manifestation of ***reactivation*** (occur by stress stimulus).
  - ***Erythema multiforme may*** be associated with HSV infection.
  - HSV-1, varicella zoster virus (VZV) may cause Bell's palsy.
- **Genital Infection (HSV-2)**
  - Widely placed bilateral lesion (vesicles, pustules, or ***painful*** erythematous ulcer) are characterstic of primary infection.
  - ***Cervix*** and ***urethra*** are most commonly involved ***in*** women with primary infection and penis in males.
  - Primary infection in patient who has prior HSV-1 infection are associated with ***less*** frequent systemic manifestation and there is faster healing of lesion.
  - A clear mucoid discharge and dysuria are characterstic of symptomatic HSV urethritis.
- **Herpetic Whitlow**
  - HSV infection of finger may occur as a complication of primary oral or genital herpes by inoculation of virus through abraded skin. Seen in doctors, dentists.
- **Eczema Herpeticum (Usually with HSV-1)**
  - Generalized eruption caused by herpes infection in children suffering from eczema.
- **Herpes Gladiatorum**
  - Mucocutaneous HSV infection of thorax, ears, face and hands.
  - Seen in wrestlers due to recurrent trauma.
- **Eye Infection (HSV-1)**
  - **MC** cause of corneal blindness in USA
  - Cause keratitis, stromal keratitis, necrotizing retinitis, chorioretinitis (in neonates and HIV patients)
- Central and peripheral nervous system manifestation

  A. Encephalitis
  - HSV -1 is ***MC*** cause of ***sporadic encephalitis***.
  - Present as acute onset of fever, focal neurologic signs especially of ***temporal lobe***.
  - ***Most sensitive non-invasive*** method for early diagnosis of HSV encephalitis is demonstration of HSV DNA in CSF by PCR **(Investigation of choice)**.
  - Demonstration in **brain tissue by biopsy** is over all most sensitive but **invasive** method.

  B. Meningitis
  - HSV is ***MC*** cause of recurrent Lymphocytic meningitis ***(Mollaret's meningitis)***.
  - HSV-2 is the usual cause
  - Diagnosed by demonstration of HSV DNA or HSV antibodies in CSF.

I

Gingivitis and pharyngitis are most frequent clinical manifestation of primary infections

I

Typical lesion of HSV: Vesicle

- Base of vesicle contains Tzank cells

- **Visceral infection**
  - **Oesophagitis**, *MC* site is **distal oesophagus.**
  - Endoscopically obtained secretion for cytology and culture provide most useful material for diagnosis.
  - Pneumonia in immunocompromised.
  - Hepatitis - may lead to disseminated intravascular coagulation.
- **Neonatal HSV infection**
  - Infection is usually acquired perinatally at the time of delivery.
  - Of all age group, neonates have highest frequency of visceral or CNS infection.
  - 70% are due to HSV-2 and 30% due to HSV-1
  - Skin lesion are the most commonly recognized feature but may not be seen in all cases.

I

Neonates have highest propensity to visceral or CNS infection

Diagnosis

- Both clinical and laboratory
- Scrapings from base of characteristic lesion is taken and stained with Wright's, Giemsa (Tzanck preparations) to detect giant cell or intranuclear inclusions. Sensitivity is low and this can't differentiate between VZV and HSV.
- HSV infection is best confirmed (specific test) by ***isolation*** of virus in tissue culture. Human diploid fibroblast is the preferred tissue.
- *PCR* for detection of HSV DNA is most sensitive. *... Harrison 19/e, p 1190, 18/e, p 1459*
- **Serology:** Type specific antibodies are useful to diagnose primary infection and asymptomatic carriers.

Treatment

- Acyclovir is most frequently used drug. Other are valacyclovir and vidarabine.

HERPES VIRUS SIMAE: B VIRUS

- Infect old world monkeys in a manner similar to the herpes simplex infection of human.
- Human cases may occur after bite of monkey or in some cases through handling of monkey tissue. Human disease is usually fatal.
- Properties are similar to H. simplex, but antibody against H. simplex are not protective.

VARICELLA - ZOSTER

- Causative agents of:
  - Varicella = Chickenpox (Primary infection)
  - Herpes zoster = Shingles (Reactivation of latent infection).

I

Varicella Zoster:
- Human are the only reservoir
- Cause chicken pox and shingles
- CNS is the MC extracutaneous site of involvement in chicken-pox

Pathogenesis

- Humans are *only* known reservoir of VZV.
- **Primary infection** *(Chickenpox)* - Transmitted by respiratory route or through conjunctiva.
- **Recurrent infection** - (Herpes zoster)- During primary infection virus infect dorsal root ganglia, where it remains latent. When immunity wanes, virus reactivates and travel along sensory nerve to produce zoster lesion on mucosa, or skin supplied by it.

Clinical manifestation

I. **Chickenpox**
- Highly contagious disease (secondary attack rate 90%) affecting 5-9 year children most commonly.
- **IP** - 10 to 21 days
- Patient **is infectious** *48 hours prior to onset of vesicular rash to until all vesicles are crusted.*
- Skin lesions - The hallmark of infection includes maculopapules, vesicles and scabs in various stage of evolution.
- Immunocompromised have severe (often hemorrhagic) and long lasting lesion with higher rate of visceral complication and fatality.
- Most common infectious complication is secondary bacterial superinfection of skin usually by S. pyogenes or S. aureus. *... Harrison 19/e, p 1194, 18/e, p 1463*

I

- Herpes zoster most frequently involves T_3-L_3
- Herpes zoster is the reactivation of latent infection of VZV

- The *MC* **site of extracutaneous** involvement in children **is CNS.**
- Varicella pneumonia is the most serious complication occurring mostly in adults.
- *Perinatal varicella* is associated with high mortality when maternal disease develop with **in 5 days before delivery or** within 48 hr **thereafter.**

II. Herpes zoster (Shingles)

- Reactivation of latent infection.
- Age group – 60 and beyond.
- Characterized by **unilateral** lesion within a dermatome associated with severe pain. The dermatome from T3 to L3 is most frequently affected.
- Patient with herpes zoster can transmit infection to seronegative individual which will develop chickenpox.
- **Zoster sine herpetica:** Zoster without skin lesion, characterized by pain in specific dermatome with seropositivity.
- **Zoster Ophthalmicus** – Due to reactivation in ophthalmic branch of trigeminal (gasserian) ganglia.
- **Ramsay Hunt syndrome** – Due to reactivation in geniculate ganglion of facial nerve.
- Most debiliating complication of Shingles is pain associated with acute neuritis and post-herpetic neuralgia.
- Patient with Hodgkin's disease and NHL are at greatest risk for progressive Herpes zoster.

I

Zoster Ophthalmicus: Reactivation of VZV infection in ophthalmic branch of trigeminal (gasserian) ganglia.

Diagnosis

- Diagnosis is usually clinical. Tzank smear shows multinucleated giant cells.
- Confirmation can be done by isolation of VZV from vesicles in tissue culture cell lines or detection of DNA by PCR.
- Serology: Most frequently used are:
  - FAMA (fluorescent antibody to membrane antigen) test
  - ELISA
  - Immunoadherant hemagglutination.
- *FAMA Test and ELISA are most sensitive.*

Treatment

- No antiviral for immunocompetent child.
- *Aspirin should be* **avoided** during episode of chickenpox as it increases risk of development of Reye's syndrome.
- **Acyclovir** therapy is recomended for adolescent and adults with chickenpox of < 24 hours duration. However it does not appear to prevent postherpetic neuralgia.
- Herpes zoster – Famciclovir and valacyclovir are more effective than acyclovir.
- **Postherpetic neuralgia** and acute neuritis:
  - Analgesics
  - Amitryptyline
  - Gabapentin
  - Lidocaine patch
  - Glucocorticoid (with concomitant antiviral therapy).
- In immunocompromised patient IV acyclovir should be given.

I

CMV

- Largest virus of herpes family
- MC pathogen complicating organ transplant
- Heterophile antibody (-ve) infectious mononucleosis is the MC manifestation
- PCR is the most sensitive technique of diagnosis.

CMV = SALIVARY GLAND VIRUS

- Characterised by enlargement of infected cell and prominent intranuclear inclusion (Owl's eye appearance).
- ***Largest virus*** of herpes family.
- *MC* organism causing intrauterine infection.
- *MC* pathogen complicating organ transplantation.

Pathogenesis

- Transmitted by sexual route, transplacental, blood transfusion, organ transplant.
- Once infected, individual carry CMV for whole life.
- Infection usually remains latent, reactivation may occur when cell mediated immunity is compromised.

Clinical feature

I. Congenital infection
- CMV infection is the most common congenital infection.
- Characterized by involvement of CNS and reticuloendothelial system.
- IUGR, Petechiae, Hepatosplenomegaly, Jaundice are ***MC*** manifestation.
- Intracerebral calcification (usually periventricular) and chorioretinitis, deafness are other important findings.

II. Perinatal infection
- Infection is acquired through birth canal at the time of delivery or through breast milk.
- Mostly asymptomatic, but may lead to interstitial pneumonia in preterm infant.

III. Beyond neonatal period in immunocompetent host
- Heterophile antibody (-)ve infectious mononucleosis is ***MC*** manifestation.
- Clinically present with malaise, protracted fever, myalgia, liver function abnormality and lymphocytosis.
- Salivary gland involvement is common and is probably chronic.
- Associated with restenosis following coronary angioplasty.

IV. Immunocompromised host

a. Organ transplant patient: – Period of maximal risk of infection – Between 1 and 4 months after transplantation.
– Retinitis is late complication.
– Transplanted organ is particulary vulnerable as a target for CMV infection, e.g. CMV hepatitis in liver transplant holder.

b. AIDS patient: – Cause retinitis (cottage and cheese appearance/pizza pie retinopathy) or disseminated disease particulary when *CD4 + < 50 - 100/µl.*

Remember: Prolong CMV infection of the Kidney does not seen to be deletorious in normal person

Diagnosis
- ***Most sensitive*** method to detect CMV in blood is **PCR.**
- Congenital infection is diagnosed by culture *(best specimen saliva and urine);* PCR. Human fibroblast are used for virus isolation. Usually 2-3 weeks are needed for culture.

Treatment
- Ganciclovir or Valganciclovir is **DOC.** Foscarnet is recommended for CMV retinitis.

EPSTEIN - BARR VIRUS

- **Causative agent of Heterophile (+)ve infectious mononucleosis** (Kissing disease) = Glandular fever.
- **Associated with:** – Nasopharyngeal Ca
  – Burkitt's lymphoma
  – Hodgkin's disease (mixed cellularity type)
  – B cell lymphoma in patients with immunodeficiency
  – Fatal lymphoproliferative disorder in patient of Duncon disease
  – CNS lymphoma in AIDS patient and transplant recipient
  – Increase risk of thymoma, tonsillar and gastric carcinoma.
  – Oral hairy leukoplakia
  – Chronic fatigue syndrome

Pathogenesis
- Source of infection is usually salivary secretions, so ***kissing*** is predominate mode of transmission.
- The virus infect epithelium of oropharynx and the salivary gland; tonsillar crypts can also be infected directly. Virus then spreads through the bloodstream.
- EBV receptor CR_2/CD21 present on B cell is also receptor for C_3 component of complement; So EBV infection immortalise B cell, at least in vitro.
- Memory B cell are reservoir of EBV in body.
- Cellular immunity is more important than humoral immunity in controlling EBV infection.
- *If T cell immunity decreased, infected B cells begins to proliferate hence producing lymphoma.*

I

EBV
- Cause heterophile antibody positive infectious mononucleosis
- Salivary secretions are the predominant mode of infection
- Diagnosed by heterophile antibody test (paul Bunnel test)

I

B-lymphocytes are the principal and essential cell infected through attachment of the major viral envelope glycoprotein gp 350/220 to C-BV receptors (CD-21) ...*Ref. Greenwood, 18/e, p434*

Clinical features

- Most EBV infection in infant and young children are asymptomatic; Second peak occurs in adults
- In adolescent, most infection present as **infectious mononucleosis**.
  - IP: 4-8 weeks
  - ***MC* symptom** sore throat
  - ***MC* sign** - Lymphadenopathy (*mostly of posterior cervical nodes*)
  - Erythema nodosum, erythema multiforme may also occur.

Diagnosis

- During initial phase there is leucopenia, which is followed by increase in TLC; Lymphocytosis with ***> 10%*** atypical lymphocytes.
  - Atypical lymphocytes are ***mainly CD8 + cells*** which have undergone blast transformation.
- Thrombocytopenia
- Serological testing
  - Heterophile test ***(Paul Bunnel test)*** is used for diagnosis of IM in children and adults. Titer of 40-fold or greater is diagnostic in patient having symptoms. Titer remains positive for 3 months. Commercially available monospot test is more sensitive.
    False positive monospot test are seen in patient with connective tissue disorder, lymphoma, viral hepatitis, malaria.
  - EBV specific antibody test: ***IgM antibody to VCA*** is most useful for diagnosis of IM. Used in patients with atypical presentation or in those who lack heterophile antibody.
  - Nucleic acid hybridization is ***the most sensitive*** means of detecting EBV in patient materials.

Serology in EBV infection						
	Anti-VCA				Anti-EA	
Condition	*Heterophile*	*IgM*	*IgG*		*EA-D*	*EA-R*
Acute infectious mononucleosis	+	+	++		+	–
Convalescence	±	–	+		–	±
Past infection	–	–	+		–	–
Reactivation with immunodeficiency	–	–	++		+	+
Burkitt's lymphoma	–	–	+++		±	++
Nasopharyngeal carcinoma	–	–	+++		++	±

VCA viral capsid antigen; EA, early antigen; EA-D antibody, antibody to early antigen in locally diffuse pattern in nucleus and cytoplasm of infected cells; EA-R antibody, antibody to early antigen restricted to the cytoplasm.

Complication

- Most cases are self limited.
- ***Most deaths*** which occur very rarely are due to ***CNS complications.***
- Coombs (+)ve autoimmune hemolytic ***anemia***.
- Acute EBV may be associated with ***Guillain-Barre' syndrome, CN palsy (MC-facial nerve).***

Herpes Virus Type 6

- Also called as human ***B-lymphotropic*** virus. Infect ***CD4 + T cells***
- Cause common childhood illness exanthem subitum (sixth disease)
- In older age groups it has been associated with infectious mononucleosis syndrome, focal encephalitis.

HHV 7

- Isolated from some cases of exanthem subitum.

HHV 8

- Classified in rhadinovirus genius
- Infects dividing B cells ...*Ref. Greenwood, 18/e, p443*
- Associated with Kaposi sarcoma, Body cavity-associated B-lymphoma/multicentric Castleman's disease.

POX VIRUS

Largest pathogenic virus of vertebrates

Important pox virus are:
- Variola (causative agent of smallpox)
- Vaccinia (Artificial virus which was used as smallpox vaccine)
- Molluscum contagiosum.

I

Pox Virus
- Include
  Variola: small Pox
  Vaccinia: Small pox vaccine
  Molluscum contagiosum

VARIOLA

- *Brick shaped* enveloped ds DNA virus
- Elementary bodies are called Paschen bodies.

VACCINIA

- It is an artificial virus whose genome can accommodate about 25000 foreign base pairs.
- Eosinophillic inclusion bodies called Guarnieri bodies can be demonstrated in stained preparation.
- But it is not suitable as a vector for human use due to its pathogenic effects.
- Properties are similar to variola.

Smallpox

- On 8th May 1980 WHO anounced global eradication of smallpox.
- In India last case was found in 1975.
  Note: *Disease had been eradicated, so clinical features etc are rarely going to be asked.*

Cultivation of Poxvirus

- Both Variola and Vaccinia grow on **CAM** producing pocks.
  - Variola pocks are small, shiny, white convex, non-necrotic, non-hemmorhagic lesions with *ceiling temperature* (highest temperature above which pocks are not produced) of *38°C*.
  - Vaccinia pocks are larger, irregular, flat, greyish, hemorrhagic and Necrotic with ceiling temperature of *41°C*.
- *On tissue culture*
  - Cytopathic effect are produced by Vaccinia in 24 - 48 hours and more slowly by Variola.
  - *Inclusion bodies* called **Guarnieri bodies** can be seen.

MOLLUSCUM CONTAGIOSUM

- *Most common human disease resulting from pox virus. ...Harrison 18/e, 1476*
- Out of the four types (MCV 1 to MCV 4), type 1 is most prevalent
- Mollusccum contagiosum virus is an obligate human pathogen.
- Usually seen in children and young adults.
- Characterised by ***pink or pearly white nodules*** on skin which show large ***inclusion bodies*** called ***Molluscum bodies***.
- Virus ***cannot be grown in*** eggs, tissues culture or animals.

ADENOVIRUS

- Space vehicle (Hexagonal shape) shaped, non-enveloped virus containing *ds DNA*.
- They have capacity to carry DNA upto 7 kb so, are potential vectors of gene therapy.

Classification

- There are about 50 serotype. Type 1-7 illness account for most disease cusually respiratory illness. ..... *Ananthanarayan, 8/e, p 479*
- Types 1, 2, 5 and 6 are more commonly associated with endemic infections.
- Types 3, 4 and 7 are more in epidemic.
- Human adenovirus have been divided into 6 subgenera on the basis of DNA homology.

I

Adenovirus
- Ds DNA containing enveloped virus
- Potential candidate for gene therapy
- Upper respiratory tract infection is MC Manifestation.

Clinical manifestation

- *MC* **is upper respiratory tract infection** with rhinitis in pediatric age group
- In adults MC is acute respiratory disease caused by types 4, 7

Syndromes	Principal serotypes
Respiratory disease in children	1, 2, 5, 6
Sore throat, febrile cold, pneumonia	3, 4, 7, 14, 21
ARD in military recruits	4, 7, 21
Follicular (swimming pool) conjunctivitis	3, 7
Epidemic keratoconjunctivitis (ship-yard eye)	8, 19, 37
Diarrhea	40, 41
Hemorrhagic cystitis	11, 21

Generalized exanthem, mesenteric adenitis and intussusception are other manifestation.

Lab diagnosis

- Isolation of virus from throat, eye, urine or feces.
- It grows only in tissue cultures of human origin, e.g. human embryonic kidney, Hela or HEP-3.
- All mammalian adenovirus share a common complement fixing antigen which is detected by immunofluorescence or ELISA.

ADENO ASSOCIATED VIRUS – (DEPENDOVIRUS)

- These are virus which can multiply only in cells infected with adenovirus as they lack enough DNA.
- It is classified under family parvoviridae.

PARVO VIRUS

- Non enveloped *SS DNA* virus
- Most parvo virus are pathogenic to animals. Human pathogen is **B-19**, the causative agent of **fifth disease**.

Pathogenesis and Clinical Manifestation

- Most of the severe manifestation of B-19 viremia are due *to its ability to lyse erythroid precursor.*
- **Erythema Infectiosum (fifth disease)**
  - *MC* manifestation of B-19 infection.
  - Child present with facial rash (slapped cheek appearance) which is preceded by low grade fever.
- **Arthropathy**
  - In *adults* B-19 infection *most commonly* present as acute arthralgia and arthritis which is symmetrical and *involves wrist (most often), knee.*
- **Transient aplastic crisis**
  - B-19 infection is the *MC cause of transient aplastic crisis in patient* with chronic hemolytic disease.
  - Unlike patient with erythema infectiosum or arthropathy, these patients can readily transmit B-19 infection to other people.
- **Immunodeficient patient**
  - *MC* manifestation is chronic anemia with reticulocytopenia (Pure red cell aplasia)
- **Fetal and congenital infection**
  - Maternal B-19 infection usually does not adversely affect fetus but can rarely cause nonimmunohydrops fetalis if infection occur in first 20 weeks of pregnancy.

Diagnosis

- Most commonly relies on B-19 specific IgM and IgG antibodies.

I

Parvo virus
- Non-enveloped SS DNA-virus
- B-19 is one of the few parvo virus which are pathogenic to humans
- B-19 causes
  - Fifth disease
  - Transient aplastic crisis

PAPOVA VIRUS

- Nonenveloped, Icosahedral double stranded DNA, tumor viruses
- Family contains 2 genera:
  1. ***Polyoma virus*** – which contains SV 40, polyoma viruses.
  2. ***Papilloma virus*** – which contain human and animal papilloma virus.

HUMAN PAPILLOMA VIRUS (HPV)

- HPV ***selectively infects*** the epithelium of skin and mucous membrane and may immortalize the keratinocyte leading either asymptomatic infection or warts or neoplasia.
- Genome consists of: - Early (E) region
  - Late (L) region
  - Upstream regulatory region (URR).
- Products of E genes **(E6; E7)** are related to immortalization or malignant transformation of keratinocytes by interfering with **P53, Rb gene** respectively.
- HPV ***infects only*** human skin and ***grows only*** in organ cultures of human skin.

I

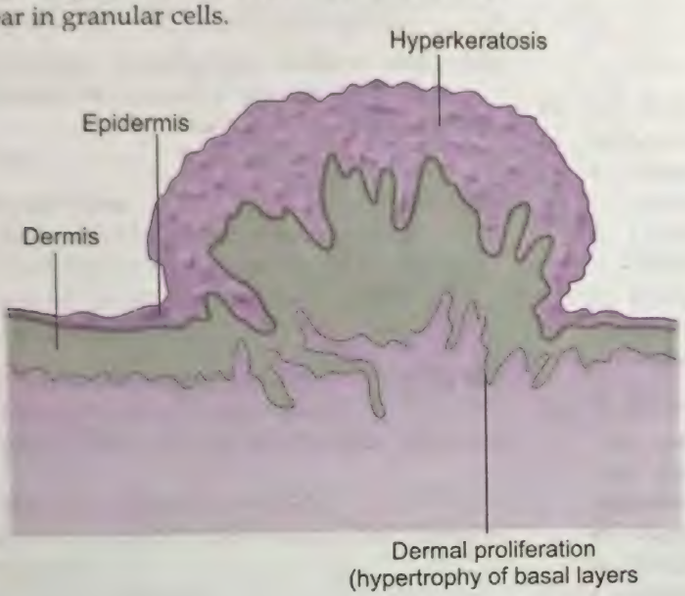
Human papilloma virus
- Member of papova virus family
- Non-enveloped DNA virus
- Selectively infects the epithelium of skin and mucous membranes where it immortalize the keratinocytes

Clinical features

- Replication of HPV begins with the infection of ***basal cells***.
- ***Koilocytes*** appear in granular cells.

I

HPV-16 is the commonest HPV associated with Ca cervix



Clinical Presentation

a. **Cutaneous Warts**
   - Common Warts (verruca vulgaris) – Type 1, 2, 3, 4
   - Plantar warts (verruca plantaris)
   - Flat warts (verruca plana) – *MC* among children – Type 3, 10
   - Also associated with Sq cell carcinoma and dysplasia of penis, anus, vagina and vulva; epidermodysplasia verruciformis (type 5, 8).
   - HPV-7 is particularly seen in Butchers and Fishmongers

b. **Anogenital warts (condyloma acuminatum)**
   - Caused by type 6 and 11
   - In women vulva & vgina are commonest site.
   - In male, the most common site of lesion are: Shaft of penis, peri-anal skin and the anal canal.

c. **Orolaryngeal lesion**
   - Recurrent respiratory papillomatosis: Caused by HPV type 6 & 11. Child acquire infection while passage through birth canal
   - Adult acquire infection from orogenital contact with an infected sexual partner
   - Disease is characterized by presence of benign squamous papillomata on the mucosa of the respiratory tract, most commonly on the larynx.
   - Surgery is the only treatment and recurrence is common Oral papillomatosis is the other disease caused by variety of HPV infections.

d. **Neoplastic lesion**
   - CIN-1: – Type 6, 11 (low risk)
   - Cervical cancer – 16, 18, 31, 33, 45 (high risk types)
   - Squamous cell Ca – HPV types 5 or 8 in patients of epiderm
   - odysplasia verruciformis
   - Oropharyngeal squamous cell Ca

Diagnosis

- The ***most sensitive*** and ***specific*** method of diagnosis is PCR or hybrid capture assay to detect HPV nucleic acids and to identify specific virus type.

Treatment

- ***Cryosurgery*** is initial *treatment of choice* for condyloma accuminatum.
- Topically
  - Podophyllum, podofilox
  - Interferon (IFN)

Prevention

- **Two vaccines are available:** Quadrivalent (Gardasil, Merck) and bivalent (Cervarix) vaccines.

Multiple Choice Questions

Herpes Virus

1. **The most common cause of sporadic viral encephalitis is:** [AIIMS 04]
   a. Japnese B encephalitis
   b. Herpes simplex encephalitis
   c. HIV encephalitis
   d. Rubeola encephalitis

2. **A neonate develops encephalitis without any skin lesions; most probable causative organism is:**
   a. HSV I b. HSV II [AIIMS 02]
   c. Meningococci d. Streptococci

3. **True about herpes virus:** [PGI 03]
   a. HSV encephalopathy is treated with acyclovir
   b. Oropharyngeal involvement is common in **HSV-1**
   c. Recurrent genital involvement is seen in HSV-2
   d. Recurrence is rare in HSV-1

4. **Regarding HSV-2 infection true is / are:** [PGI 02]
   a. Primary infection is usually widespread
   b. Recurrent attacks are due to reactivation of latent infection
   c. Encephalitis can be caused by HSV-2
   d. Newborn may acquire infection via the birth canal at the time of labour
   e. Treatment is with acyclovir

5. **A 29-year-old person comes with focal seizures. MRI shows frontal and temporal enhancement. What is the most probable diagnosis:** [AI 10]
   a. Meningococcal meningitis
   b. Herpes simplex encephalitis
   c. Japanese encephalitis
   d. Enterovirus encephalitis

Varicella Zoster Virus

6. **Infectivity of chickenpox lasts for:**
   a. Till the last scab falls off [AI 02, AIIMS 00]
   b. 6 days after onset of rash
   c. 3 days after onset of rash
   d. Till the fever subsides

7. **Which of the following pair is correct?** [PGI 05]
   a. RSV – Bronchiolitis
   b. HHV5 – Infectious mononucleosis
   c. Parvovirus exanthem subitum
   d.HHV-6 - Kaposi sarcoma
   e. VZV – Chickenpox

8. **Varicella Zoster remains latent in:** [AI 10]
   a. Trigeminal Gangilion b. Macrophages
   c. T-cells d. B-cells

CMV

9. **Which of the following does not establish a diagnosis of congenital CMV infection in a neonate?** [AI 03]
   a. Urine culture of CMV
   b. IgG CMV antibodies in blood
   c. Intra-nuclear inclusion bodies in hepatocytes
   d. CMV viral DNA in blood by polymerase chain reaction

10. **All of the following statement are true regarding CNS infection except:** [AIIMS 04]
    a. Measles virus is causative agent of subacute sclerosing panecephalitis
    b. Cytomegalovirus causes bilateral temporal lobe hemorrhagic infarction
    c. Prion infection cause spongiform encephalopathy
    d. JC virus is causative agent of progressive multifocal leucoencephalopathy

11. **A 40-year-old man underwent kidney transplantation. Two month after transplantation, he developed fever and feature suggestive of bilateral diffuse interstitial pneumonia. Which of the following is most likely etiologic agent?** [AIIMS 03]
    a. Herpes simplex virus b. Cytomegalovirus
    c. Epstein-barr virus d. Varicella - zoster virus

12. **A patient has undergone a renal transplantation 2 months back and now presented with difficult breathing. X-ray showed bilateral diffuse interstitial pneumonia. The probable etiologic agent would be:** [AIIMS 02]
    a. CMV b. Histoplasma
    c. Candida d. Pneumocystic carinii

13a. **Renal involvement is seen in which of the following infections?** [PGI 03]
    a. Cytomegalovirus
    b. Polyoma virus
    c. Human papilloma virus
    d. HIV
    e. HBV

13b. **In a patient with a vesicle on shin. Microscopy on Tzank smear showed giant cells. Causative agents is:** [AIIMS 15]
    a. Vaccinia virus
    b. Varicella zoster
    c. Tuberculous
    d. Molluscum contagiosum

EBV

14. **The following diseases are associated with Epstein - Barr virus infection, except:** [AI 06]

a. Infectious mononucleosis
b. Epidermodysplasia verruciformis
c. Nasopharyngeal carcinoma
d. Oral hairy leukoplakia

15. **Epstein Barr (EB) virus has been implicated in the following malignancies except:** [AI 04]
a. Hodgkin's disease
b. Non-hodgkin's lymphoma
c. Nasopharyngeal carcinoma
d. Multiple myeloma

16. **Epstien Barr virus causes all the following except:**
a. Infectious mononucleosis [AI 02; AIIMS 99]
b. Measles
c. Nasopharyngeal carcinoma
d. Non-Hodgkins lymphoma

17. **A patient with sore throat has a positive Paul Bunnel test. The causative organism is:** [AI 00]
a. EBV b. Herpes virus
c. Adenovirus d. Cytomegalovirus

18. **Epstein-Barr virus causes autoimmunity by:**
a. Molecular mimicry [AI 2012]
b. Inducing inappropriate expression of class II MHC
c. Release of sequestere antigens
d. Polyclonal B cell activation

19. **Epstein Barr virus is associated with:** [AIIMS 04]
a. Carcinoma larynx
b. Carcinoma bladder
c. Carcinoma nasopharynx
d. Carcinoma maxilla

20. **EBV associated with:** [PGI 03]
a. Ca tonsil b. Nasopharyngeal Ca
c. Anal Ca d. Infectious mononucleosis

21. **Disease caused by Epstein Barr virus are:**
a. Infectious mononucleosis [PGI 02; 01]
b. Burkitt's lymphoma
c. Kaposi sarcoma
d. Nasopharyngeal Ca
e. Herpangina

Others

22. **Hypoplasia of limb and scarring is caused by:** [AIIMS May 2011]
a. Varicella b. Herpes simplex
c. Rubella d. Toxoplasma

23. **Virus causing hemorrhagic cystitis, diarrhea and conjunctivitis is:** [AI 01]
a. RSV b. Rhinovirus
c. Adenovirus d. Rotavirus

24. **Viral enterotoxin is detected as a possible mechanism of pathogenesis in:** [AI 98, PGI 02]
a. Adenovirus
b. Rotavirus
c. Calcivirus
d. Astrovirus

25. **Parvovirus B-19 does not cause:** [AIIMS 08]
a. Roseola infantum
b. Aplastic anemia in sickle cell
c. Fetal hydrops
d. Collapsing FSGS

26. **Parvo virus causes:** [PGI 07]
a. Aplastic anemia b. Erythema infectiosum
c. Roseola infantum d. Arthritis

27. **In parvovirus infection what is common in adult:**
a. Bone marrow [PGI 07]
b. PRCA
c. Erythema infectiosum
d. Arthropathy

28. **Which of the following statement is correct:** [PGI 05]
a. Viral warts usually resolve spontaneously
b. Plantar warts should not be excised
c. Callosity are formed occupationally
d. Corns are viral in etiology
e. Plantar warts are painless

29. **Vaccine preparation requires which virus as vector:** [PGI 01]
a. Rhinovirus b. Vaccinia
c. Adenovirus d. Ebola
e. Hepatitis B

30. **HPV vaccine is:** [AIIMS 09]
a. Monovalent b. Bivalent
c. Quadrivalent d. Both b and c

31. **About parvovirus B19 all are true except** [AI 2011]
a. Spread by respiratory route
b. Has affinity for erythrocyte progenitor cells
c. Causes transient aplastic crisis
d. Transplacental transfer occurs in only 10% of cases

32. **Virus causing head and neck cancer:** [PGI Nov. 11]
a. EBV b. HSV
c. HPV d. HBV
e. HCV

33. **Not a cause of epidemic encephalitis:** [PGI May 2013]
a. Herpes simplex virus
b. Rabies
c. West Nile virus
d. Nipah virus
e. Japanese encephalitis virus

34. **Post transplant nephropathy after 1 month is most likely be due to?** [AIIMS May 2014]
a. Hepatitis C
b. HHV-6
c. Polyoma BK virus
d. Herpes simplex viruses

Explanations and References with Illustrative Answers

1. **Ans. (b) Herpes simplex encephalitis** *Ref. Harrison 19/e, p 891, 18/e, p 3421*

"The most common virus causing sporadic cases of encephalitis in immunocompetant adult are HSV-1, VZV, EBV and less comonly enterovirus."

Remember: Epidemic of encephalitis are caused by Arbovirus: **1. Alphaviruses, 2. Toga viruses, 3. Bunyaviruses**

Virus causing encephalitis			
• ***Common:***	– *Arbovirus*	– *Enterovirus*	– *EBV*
	– HSV-1	– Mumps	– VZV
• **Less common:**	– CMV, HIV, measles		
• **Rare:**	– Adenovirus, influenza virus, parainfluenza virus, rabies, rubella		

Neurological manifestation of Herpes			
CNS manifestation		*ANS manifestation*	*PNS manifestation*
Encephalitis (HSV-1 > HSV-2)	Involving temporal lobe especially	• ANS dysfunction especially of sacral region leading to numbness, tingiling of the buttocks or perineal areas.	• Bell's palsy • Cranial polyneuritis
Meningitis (HSV-2 > HSV-1)	HSV is *MC* cause of recurrent lymphocytic meningitis ***(Mollaret's meningitis)***	• Urinary retention, constipation, impotence. • **Guillain-Barre' syndrome**	

2. **Ans. (b) HSV-II** *Ref. Harrison 18/e, p 1458, 19/e p 1180; Greenwood 18/e, p 426*

Although skin lesion are the most common recognized feature of disease, many infants do not develop lesion until well into the course of disease.

Most cases of neonatal infection are due to HSV-2.

So, it can be said that initially skin lesions may not be present in case of HSV -2 encephalitis.

Meningococci do not cause encephalitis in neonates.

Neonatal herpes

HSV-2 > HSV-1

Skin, eye and mucous membrane disease	**Disseminated disease**	**Encephalitis**
– 10-12 days postpartum	– Around 6th day	– Manifest around 2-3 weeks
– Vesicular lesions on skin (may be absent)	– Characterized by pneumonitis & hepatitis	

Remember: Of all HSV infected individual, neonates have highest frequency of visceral and/or CNS infection. *Neonatal infection is usually acquired perinatally from contact with infected genital secretions at the time of delivery. Risk is greatest when mother has primary HSV infection at the time of delivery.*

3. **Ans. (a), (b) and (c) HSV encephalopathy is treated with acylovir, Oropharyngeal involvement is common in HSV -1, and Recurrent genital involvement is seen in HSV-2** *Ref. Harrison 19/e, p 1176 - 1177, 18/e, p 1453*

Difference between HSV 1 and HSV 2	
HSV Type 1	**HSV Type 2**
• Cause lesion in and around mouth	Cause lesion around genital area
• Transmitted by direct contact or droplet spread	Usually transmitted sexually
• Replicate poorly in chick embryofibroblast cell	Replicate well
• Relatively sensitive to antiviral agents	Resistant
• Less neurovirulent	More neurovirulent
• Infectivity is less temperature sensitive	More temperature sensitive

Remember: – Genital HSV-2 infection is twice as likely to reactivate and recurs 8 to 10 times more frequently than genital HSV-1 infection
– Similarly oral-labial HSV-1 infection recurs more frequently than oral-labial HSV-2 infection.
– The overall genomic sequence homology between HSV-1 and HSV-2 is 50%

Treatment of CNS infection: HSV encephalitis: IV acyclovir for 10 days.

4. **Ans. (a), (b), (c), (d), (e) (All are correct options)** *Ref. Harrison 19/e, p 1177 - 1179, 18/e, p 1455 - 1457,*

Characteristic of HSV-2 infection:

- Usually (but not absolutely) cause lesion below waist.
- First episode of primary genital herpes is characterised by fever, headache, malaise, myalgia.
- Widely, spaced bilateral lesions on external genitalia are characteristic.
- Cervical and urethral involvement seen in >80% woman in 1st episodes.
- The 12-month recurrence rate among patient after first episode HSV-2 and HSV-1 infections are 90% and 55% respecitvely.
- **Neonatal HSV infection:** Usually acquired perinatally at the time of delivery.
- **Treatment:** Acyclovir is most commonly used drug.

5. **Ans. (b) Herpes simplex encephalitis** *Ref. Harrison 18/e, p 1457, 19/e p 1178*

Herpes simplex encephalitis

- In children and young adults primary HSV infection can led to encephalitis, by neurotropic spread of virus from periphery via olfactory bulb.
- Reactivation of latent CNS infection is another mechanism for the development of HSV encephalitis.
- In other majority of cases, there is prior mucocutaneous HSV-1 infection which gets reactivated.
- **Clinical hallmark** of HSV infection include acute onset of fever, and ***focal neurological symptoms and signs, especially of temporal lobe.***

Diagnosis	– CSF protein and CSF lymphocytosis
	– Brain biopsy is gold standard
	– HSV DNA detection in CSF by PCR has largely replaced biopsy
Treatment	– IV acyclovir

6. **Ans. (b) 6 days after onset of rash** *Ref. Park 22/e, p 136*

"Period of communicability of varicella range from 1 to 2 days before the appreance of rash, and 4 to 5 days there after."
The patient ceases to be infectious once the lesions have crusted.
Scabs are not infective
So, ***option*** "**a**" is wrong.

Important features of chickenpox

Causative agent	– Varicella zoster virus (HHV type 3)
Incubation period	– 14 to 16 days
Secondary attack rate	– 90%

Rash	
Chickenpox	***Smallpox***
– Superficial	Deep seated
– Pleomorphic	Only one stage of rash at 1 time
– Centripetal	Centrifugal
– Unilocular	Multilocular
– Dew drop like appearance	Umbilicated
– Inflammation (+)nt	No area of inflammation around vesicle
– Mostly flexor surface	Mostly extensor surface

7. **Ans. (a), (b) and (e) RSV – Bronchiolitis, HHV5 – Infectious mononucleosis, and VZV – Chickenpox** *Ref. Ananthanarayan 9/e, p 471*

Remember: ***RSV*** is most common cause of bronchiolitis.
Other causes are: – Parainfluenza virus – Adenovirus
– Influenza virus – Mycoplasma pneumoniae

8. **Ans. (a) Trigeminal ganglion** *Ref. Ananthanarayan 8/e, p 473, 9/e, p 472*

Site of latency of varicella virus
- Dorsal root ganglia (Most frequent) T3-L2
- Trigeminal ganglia (frequently ophthalmic branch)

9. **Ans. (b) IgG CMV antibodies in blood** *Ref. Nelson 17/e, p 1068; Greenwood 18/e, p 440*

"IgG antibody test is of little diagnostic value as positive results also reflects maternal antibodies" although its absence exclude the diagnosis of congenital CMV infection."
.... Nelson

Diagnosis of congenital CMV infection
- **Virus isolation**
  - Definitive and best method.
  - Culture: Urine and saliva are best specimen for culture although it can be isolated from buffy coat (blood), bronchoalveolar washing.
  - PCR: Replaced virus isolation for routine detection of CMV infection.
- **Antibody assay**
  - IgG test are not diagnostic as positive results reflects maternal antibodies.
  - IgM test lacks sensitivity and specificity and are unreliable for diagnosis of congenital infection.
- **Detection in amniotic fluid**
  - Fetal infection can be confirmed by viral isolation from amniotic fluid.
  - Detection of viral genome by PCR in amniotic fluid is equally sensitive (Viral genome > 10^5 genome is a predictor of symptomatic congenital infection).

Remember: CMV infected cells contain large intranuclear and smaller intracytoplasmic inclusions which are pathognomic for CMV infection.

10. **Ans. (b) CMV virus causes B/L temporal lobe infarction** *Ref. Harrison 19/e, p 1192, 18/e, p 1473-1474*

CNS manifestation of CMV:
- CMV rarely cause CNS infection.
- Two forms of CMV encephalitis are seen:
  1. Resemble HIV encephalitis and present as progressive dementia.
  2. Ventriculoencephalitis—Characterized by cranial nerve deficit, nystagmus and ventriculomegally.
- In immunocompromised patient CMV can also cause subacute progressive polyradiculopathy.

No where is given that CMV can cause temporal lobe infarction: Hence answer

Other options:
- **Subacute sclerosing panencephalitis**
  - It is a rare chronic progressive demyelinating disease of CNS associated with a chronic permissive infection of brain tissue with measles virus.
- **Progressive multifocal leucoencephalopathy**
  - Progressive disorder characterised pathologically by multifocal areas of demyelination of varying size distributing throughout the CNS caused by JC virus.
- **Spongiform encephalopathy**
  - Caused by prion infection; HIV infection.

11. **Ans. (b) Cytomegalovirus** *Ref. Harrison 19/e, p 1192, 18/e, p 1124*

It is a case of diffuse interstitial pneumonitis due to CMV.

Remember:
- **CMV** is the **MC** infection complicating organ transplantation.
- **CMV** is **MC** cause of intrauterine infection.
- Risk of post-transplant CMV infection is greatest 5-13 weeks after transplant

Infections after Kidney Transplantation			
	Period after transplanatation		
Infection site	***Early (< 1 month)***	***Middle (1 - 4 months)***	***Late (> 6 months)***
Urinary tract	Bacteria (Escherichia coli, Klebsiella, Enterobac- teriaceae, Pseudomonas, Enterococcus) associated with bacteremia and pyelonephritis, Candida	Cytomegalovirus (fever alone is common) BK virus *(nephropathy, graft failure, vasculopathy)*, JC virus.	Bacteria; late infections usually not associaed with bacteremia
Lungs	Legionella	***CMV diffuse interstitial*** *pneumonitis,* Pneumocystis, Aspergillus, Legionella	Nocardia, Aspergillus, Mucor
Central nervous system		Listeria meningitis, CMV encephalitis, Toxoplasma gondii	CMV retinitis, Listeria meningitis, cryptococcal meningitis, Aspergillus, Nocardia

12. **Ans. (a) CMV** *Ref. Harrison 19/e, p 1192, 18/e, p 1124*

Already explained

13a. **Ans. (a), (d) and (e) Cytomegalovirus, HIV and HBV** *Ref. Harrison 18/e, p 2346 - 2347*

Virus causing Glomerular Disease	
Disease	***Virus***
• Focal segmental glomerulosclerosis	HIV, HBV, Parvo virus, Coxsackie
• Membrane proliferative glomerulonephritis	HBV, HCV, HIV, CMV, EBV
• Diffuse proliferative glomerulonephirits	Coxsackie virus
• Membranous nephropathy	HBV; HCV
• Endocapillary proliferative GN	Measles, Dengue
• Mesangioproliferative GN	Parvovirus, Mumps

Remember: Other infectious causes of Membraneous GN–Syphilis, Malaria, Schistosomiasis, Filariasis, Leprosy.

13b. **Ans. (b) i.e Varicella zoster** *Ref. Ananthanarayan 9/e, p 472*

"Multinucleated giant cells and type a intranculear inclusion bodies may be seen in smears prepared by scraping the base of early vesicles (Tzank smears) and stained with toludine blue, Giemsa or Papanicolou stain"

Note: Electron microscopy of vesicle fluid can demonstrate the typical virus.

14. **Ans. (b) Epidermodysplasia verruciformis** *Ref. Harrison 19/e, p 1199, 18/e, p 1467*

Epidermodysplasia verruciformis is a rare inherited disease with numerous flat warts on the hand and feet. These individuals have defect in cell mediated immunity and increased susceptibility to human papilloma virus infection.

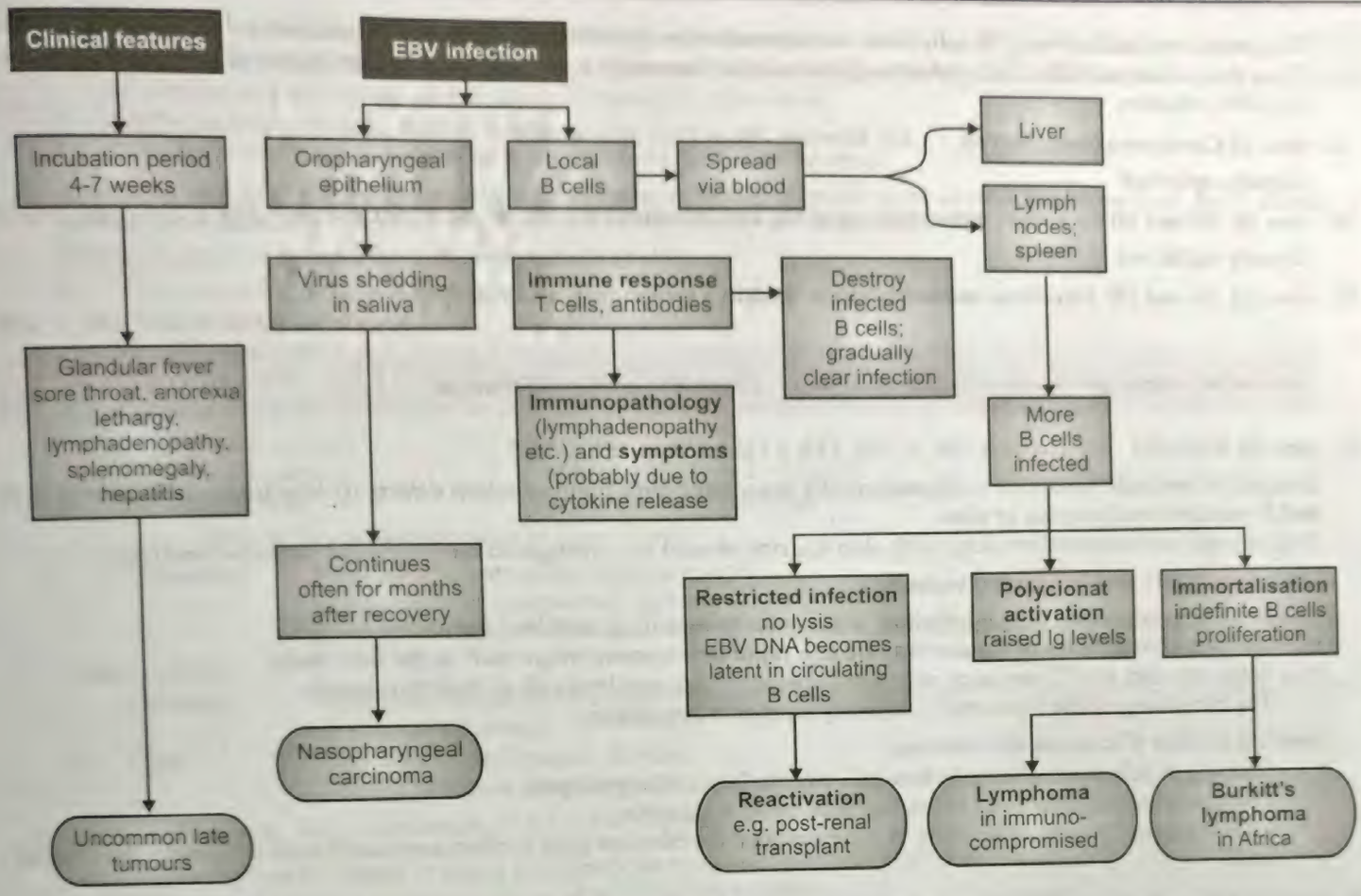
Remember: Multiple myeloma is associated with human herpes virus 8, in some cases not with EBV.

15. **Ans. (d) Multiple myeloma** *Ref. Harrison 19/e, p 1187, 18/e, p 1469*

Already explained

16. **Ans. (b) Measles** *Ref. Harrison 17/e, p 1107-1108, 18/e, p 1469*

Already explained



17. **Ans. (a) EBV** *Ref. Ananthanarayan 8/e, p 476, 9/e, p 476*

Paul Bunell test is the standard diagnostic procedure of infectious mononucleosis which is caused by EBV.

Paul Bunell test detects heterophile antibody.

Paul Bunell test

- In this test inactivated serum (56°C for 30 minutes) in doubling dilutions is mixed with equal volumes of a 1% suspension of sheep erythrocytes.
- An agglutination titre of 100 or above is suggestive of infectious mononucleosis.
- For confirmation, differential absorption of agglutinins with guinea pig kidney and ox red cells is necessary
- Infectious monoucleosis antibody is removed by ox red cell but not guinea pig kidney.
- The Paul Bunell antibody develops early during the course of infectious mononucleosis, and disappears within two months.
- ***False-positive:*** In patient with lymphoma, hepatitis, malaria, connective tissue disease.

Differential absorption test for Paul-Bunnell antibody		
	Guinea pig kidney	***Ox red cells***
Normal serum	Absorbed	Not absorbed
Antibody after serum therapy	Absorbed	Not absorbed
Infectious mononucleosis	Not absorbed	Absorbed

Remember:
- ***MC*** cause of ***heterophile antibody (+)ve*** infectious mononucleosis is **EBV**.
- MC cause of ***heterophile antibody (–) ve*** infectious mononucleosis is **CMV**.
- Heterophile (–) ve IM is also caused by toxoplasmosis, Listeria, non-infectious stimuli.

18. **Ans. (d) Polyclonal B-cell activation** *Ref. Harrison 18/e, p 1467; Jawetz 27/e, p 475*

EBV receptor (CD-21) on the surface of B-cells is also the receptor for the C3d component of complement. So, when B-cells are infected with EBV they become transformed and can proliferate indefinitely.

This polyclonal activation of B-cells leads to the production of antibodies directed against host cells and viral proteins. Thus, this is also not difficult to understand that cellular immunity is more important than humoral immunity in controlling EBV infection.

19. **Ans. (c) Carcinoma nasopharynx** *Ref. Harrison 19/e, p 1187, 18/e, p 1469*

Already explained

20. **Ans. (a), (b) and (d) Ca Tonsil, Nasopharyngeal Ca, and Infectious mononucleosis** *Ref. Harrison 19/e, p 1187, 18/e, p 1469*

Already explained

21. **Ans. (a), (b) and (d) Infectious mononucleosis, Burkitt's lymphoma, and Nasopharyngeal Ca** *Ref. Harrison 17/e, p 1107, 18/e, p 1469*

Remember: Herpangina is caused by Coxsackie virus A, Coxsackie virus B and Echovirus.

22. **Ans. (a) Varicella** *Ref. Harrison 19/e, p 1184, 18/e, p 1464; Nelson 18/e, p 1365*

Congenital varicella syndrome is characterically associated with limb reduction defects (If infection occurs prior to limb bud formation) and scarring of skin.

Any neonate with limb defect along with skin scarring should be investigated for congenital varicella syndrome.

Limb hypoplasia and congenital varicella

- Limb hypoplasia are seen if infection occurs prior to or during limb bud formation
- ***The virus has affinity to tissues that are in a rapid development stage such as the limb buds***
- Fetus infected at 6-12 weeks of gestation appears to have maximal risk of limb hypoplasia
- The remaining of the torso may be entirely normal in appearance

Scarring of Skin (Cicatrical skin lesions)

- Scarring of skin is a common feature of congenital varicella syndrome
- The characteristic cutaneous lesion has been called a ***Cicatrix***
- Cicarix represents zigzag scarring in a dermatomal distribution (and is often associated with atrophy of the affected limb)

23. **Ans. (c) Adenovirus** *Ref. Ananthanarayan 9/e, p 481; 8/e, p 481*

Adenovirus causes respiratory disease, conjuctivitis, cystitis, diarrhea

24. **Ans. (b) Rotavirus** *Ref. Harrison 18/e, p 1591*

Mechanism of Rotavirus diarrhea:

a. Rotavirus destroy mature enterocytes of proximal small intestine.
   - Loss of absorptive villi and proliferation of secretory crypt cells
     - *Secretory diarrhea*
   - Brush border enzymes are reduced so unmetabolized disaccharides accumulates resulting in:
     - *Osmotic diarrhea*

b. NSP_4 = enterotoxin which alteres epithelial cell function and permeability causing secretory diarrhea.

c. Virus evoke fluid secretion by the activation of enteric nervous system in intestinal wall.

25. **Ans. (a) Roseola infantum** *Ref. Harrison 19/e, p 1195, 18/e, p 1479*

Roseola infantum or exanthem subitum is caused by HHV-6 and HHV-7 (rarely).

Diseases caused by Parvo virus:

- Erythema infectiosum
- Seronegative arthritis
- Aplastic crisis in hemolytic anaemia
- Fetal infection leading to non-immunohydrops fetalis
- Chronic anaemia in immunocompromised
- Hemophagocytic syndrome
- Severe anaemia in malarial patients.

26. **Ans. (a), (b) and (d) Aplastic anemia, Erythema infectiosum and Arthritis** *Ref. Harrison 19/e, p 1196, 18/e, p 1479*

Already explained

27. **Ans. (d) Arthropathy** *Ref. Harrison 19/e, p 1196, 18/e, p 1479*

Arthropathy is most common manifestation of parvo virus in adults.

- Distribution of the affected joints is often symmetrical with athralgia affecting the small joints of hand and occasionally ankle knee and wrists. Resolution occurs within a few weak
- Erythema infectiosum is seen in infants
- Bone marrow aplasia is seen in patients of chronic hemolytic anaemia.

28. **Ans. (a, b) and (c) Viral warts usually resolve spontaneously, Plantar warts should not be excised, and Callosity are formed occupationally** *Ref. Harrison 17/e, p 1118; Short case by S. Das 2/e, p 20*

Warts are patches of hyperkeratotic overgrowth of skin.

- Three types of warts can be seen:
  a. Common wart
  b. Venereal wart
  c. Senile wart
- Common wart can be:
  - Verruca vulgaris = *MC* type
  - Verruca plana = Flat wart - *MC* type in children
  - Plantar wart = Verrucaplantaris - painfull

HPV is the etiological agent of these warts.

Treatment
- Most HPV lesion resolve spontaneously.
- Cryosurgery is treatment of choice.
- Surgical excision is not recommended as it leads to scarring and recurrence rate is quite high.

Other options

- **Callosity** – Superficial circumscribed yellowish white flat thickened patch of hyperkerotic material. Etiology is mostly occupational.
- **Corn**
  - Localised hyperkeratosis of skin.
  - Usually occurs at the pressure site, e.g. on sole, foot and toes.

29. **Ans. (b) Vaccinia** *Ref. Ananthanarayan 9/e, p 461; 8/e, p 462*

- Vaccinia virus is unique in that it is an artificial virus and does not occur in nature as such.
- It is used as a vector for development of recombinant vaccines.
- Its genome can accommodate 25,000 foreign base pairs.
- Genes encoding antigens of HBV, HIV, rabies and neuropeptides are inserted in it.
- However it is not useful as vector for human use due to pathogenic effect.

30. **Ans. (d) Both b and c** *Ref. Harrison 18/e, p 1483, 19/e p 1199*

- ***HPV is associated with cervical and oral cancer. In order to reduce the incidence of these potentially lethal malignancy, HPV vaccine has been introduced.***

HPV vaccines

- Directed against viruses types that cause anogenital disease and are derived from the expression of the major capsid protein(L) gene in tissue culture.
- **Currently two vaccines are available**
  1. *Quadrivalent vaccine (Gardasil, Merck):* It contains major capsid protein from low risk types 6 and 11 and high risk types 16 and 18. It is administered to girls and young women 9 - 26 years of age.
  2. *Bivalent (cervarix):* Containing HPV types 16 and 18.

Dose: 3 doses are given in 0; 2nd and 6th month

Adverse effects: Minimal consists of mild to moderate localized pain, erythema, swelling.

31. **Ans. (d) i.e. Transplacental transfer occurs in only 10% of cases.** *Ref. Harrison 19/e, p 1195, 18/e, p 1478*

Parvovirus B19

- Member of genus erythrovirus, exclusively infects humans
- Transmission occurs predominantly via respiratory route, transfusion related transmission also seen.
- *Pathogenesis*
  - B19 replicates primarily in erythroid progenitors. This specifically is due to limited tissue distribution of B19 receptor, blood groups antigen (globoside). Resulting in transient aplastic crisis.
  - Other cells bearing B-19 receptor include megakaryocytes, endothelial cells, placenta, myocardium and liver.
- Transplacental infection is seen in 30% and the risk of fetal loss is 9%. Risk of congenital infection is < 1%.

32. Ans (a, c) EBV and HPV *Ref: Harrison 18/e p 656*

Oncogenic Microbes and Parasites	
Organism	***Neoplasm***
Human papilloma virus (Papovaviridae)	Cervical, vulvar, penil cancers, squamous cell carcinoma, oropharyngeal carcinoma
HSV type 2	Cervical carcinoma
Hepatitis B virus (Hepadnaviridae)	Hepatocellular carcinoma
Hepatitis C virus (Flaviviridae)	Hepatocellular carcinoma, Lymphoplasmacytic lymphoma
HTLV – I (Retroviridae)	Adult T-cell leukemia/ lymphoma
HTLV – II (Retroviridae)	T-cell variant of hairy cell leukemia
HTLV – III (Retroviridae)	AIDS related malignancies, NHL, Kaposi sarcoma, SCC (esp of Urogenital tract), Diffuse large B-cell lymphoma Burkitt's lymphoma
Epstein barr virus (Herpesviridae)	Mixed cellularity Hodgkin's, Nasopharyngeal carcinoma (anaplastic), African Burkitt's lymphoma, Post organ transplant lymphoma, Primary CNS diffuse large B-cell lymphoma, Extranodal NK/T cell lymphoma (nasal type)
H. Pylori	Gastric Malt lymphoma, Gastric cancer
Human Herpes virus 8	Primary effusion lymphoma, Multicentric castleman's disease
Schistosoma hematobium	Bladder cancer (squamous cell)
Clonorchis	Cholongio carcinoma
Opisthorchis	Cholongiocarcinoma

33. Ans. (a, b) i.e. Herpes simplex virus, Rabies *Ref. Harrison 18/e, p 3418, 3421*

Viral encephalitis	
Sporadic	***Epidemic***
Herpes virus (HSV-1*, VZV*, EBV*, CMV)	Alpha virus (ECE virus, western equine encephalitis virus)
Rabies	Flaviviruses (West Nile virus*), Japanese encephalitis virus, Pawassan virus, St. Louis encephalitis* virus)
Mumps	Bunyaviruses (California encephalitis virus, La Crosse virus)
Enterovirus	Nipah virus

* Common cause

Nipah virus

- Nipah virus infection is a newly emerging zoonosis that causes severe disease in both animals and humans. The natural host of virus are fruit bats.
- Clinical presentation of nipah virus range from asymptomatic infection to acute respiratory syndrome to fatal encephalitis.
- It can cause in pig and other domestic animals too.
- It was associated with epidemic of encephalitis in Bangladesh in 2004.

34. Ans. (c) i.e. Polyoma BK virus *Ref. Harrison 18/e, p 1126; 1128*

Causes of nephropathy (1 month after renal transplant)
CMV infection (MC) > BK virus infection > JC virus infection

Both BK virus and JC virus belongs to papova viridae. *(For details see answer 11)*

Chapter Review

1. **Most sensitive test for diagnosis of infectious mononucleosis:** [Kerala 90] [SGPGI 09]
   a. Monospot test
   b. Paul Bunnel test
   c. Lymphocytosis in peripheral smear
   d. Culture of the virus *[Ref. Harrison 19/e, p 1189]*

- Monospot test is more sensitive than classic heterophile test. it is 75% sensitive and 90% specific. False-positive monospot test is seen in connective tissue disease, lymphoma, viral hepatitis and malaria
- Nucleic acid hybridization is most sensitive means of detecting EBV in patient
- IgM antibody to VCA is most useful test for diagnosis of infectious mononucleosis

2. **Herpes simplex virus is:** [AI 91]
   a. Single stranded DNA
   b. Double stranded DNA
   c. Single stranded RNA
   d. Double stranded RNA
   [Ref. Ananthanarayan 9/e, p 466]

Herpes virus produces intranuclear type A inclusion bodies. On chick embryo CAM it produces non-necrotic pocks. Cytopathic changes include well defined foci with heaped up cells and syncytial and giant cell formation.

3. **Which of the following is not a pox virus:** [AIIMS 92]
   a. Cow pox
   b. Molluscum contagiosum
   c. Smallpox
   d. Chickenpox
   [Ref. Ananthanarayan 8/e, p 471, 9/e, p 461]

4. **Epstein Barr virus has:** [Delhi 08]
   a. Double stranded DNA virus
   b. Single stranded DNA virus
   c. Double stranded RNA virus
   d. Single stranded RNA virus
   [Ref. Ananthanarayan 8/e, p 467, 9/e, p 466]

5. **Rash pattern in chickenpox is:** [RJ 06]
   a. Centripetal
   b. Centrifugal
   c. Localized
   d. All
   [Ref. Ananthanarayan 8/e, p 472, 9/e, p 471]

6. **Paul Bunnel test is done is:** [RJ 04]
   a. Malta fever
   b. Typhus fever
   c. Enteric fever
   d. Infectious mononucleosis
   [Ref. Ananthanarayan 8/e, p 477, 9/e, p 476]

7. **Which disease has viral etiology:** [RJ 04]
   a. Myeloma
   b. Nasopharyngeal Ca
   c. Sarcoidosis
   d. Hemophilia
   [Ref. Ananthanarayan 8/e, p 476, 9/e, p 476]

8. **Genital Herpes simplex can be diagnosed by:** [AP 07]
   a. Grams stain
   b. KOH preparation
   c. Tzank smear
   d. Acid fast stain
   [Ref. Ananthanarayan 8/e, p 470, 9/e, p 470]

9. **True about virus is:** [UP 00]
   a. HSV-I causes encephalitis
   b. EBV affects T lymphocyte
   c. CMV is always symptomatic
   d. Herpes zoster is not reactivated
   [Ref. Ananthanarayan 8/e, p 470, 9/e, p 469]

10. **Which virus remains dormant but reactivate is:** [UP 00]
    a. Herpes simplex
    b. Herpes zoster
    c. EB virus
    d. CMV
    [Ref. Ananthanarayan 8/e, p 473, 9/e, p 472]

11. **Kaposi sarcoma is caused by:** [UP 01]
    a. EBV
    b. Parvovirus
    c. Herpes virus
    d. Rotavirus
    [Ref. Ananthanarayan 8/e, p 477, 9/e, p 477]

Kaposi Sarcoma is caused by HHV-8

12. **Human papilloma virus contains:** [DNB 07]
    [Ref. Greenwood 18/e 452]
    a. SSRNA
    b. DSDNA
    c. SSRNA
    d. SSDNA

13. **Zoster ophthalmicus is due to:** [DNB 12]
    a. Primary herpes infection of eye
    b. Herpes reactivation in optic nerve
    c. Herpes reactivation in trigminal nerve
    d. Herpes infection of eye in immunocompromised patient
    [Ref. Harrison 19/e p 1184]

Answers 1. a. Monospot ... 2. b. Double ... 3. d. Chickenpox 4. a. Double 5. a. Centripetal
6. d. Infectious 7. b. Nasopha... 8. c. Tzank 9. a. HSV-I cause ... 10. b. Herpes ...
11. c. Herpes ... 12. b. DSDNA 13. c. Herpes reactivation in trigminal

14. Which virus reactivate and involves the eyes: **[Jharkhand 04]**

a. Herpes-zoster
b. CMV
c. EM virus
d. Enterovirus - 70

[Ref. Ananthanarayan 9/e, p 472]

15. All are true regarding cytomegalovirus except:

a. It is DNA virus' **[Kolkata 05]**
b. Most commonly infected in the last trimester
c. Diagnosed by increased IgA in fetal blood
d. Most common cause of congenital viral infection

[Ref. Ananthanarayan 8/e, p 474, 9/e, p 473]

16. The Epstein Barr virus is implicated in all of the following except: **[DNB 05]**

a. Nasopharyngeal Ca
b. Burkitt's lymphoma
c. Infectious mononucleosis
d. Leukemia

[Ref. Harrison 18/e, p 1188, 18/e, p 1469]

17. The following about Epstien Barr virus are true except: **[MP 06]**

a. It is a member of herpes virus family
b. It infects epithelial cells of oropharynx
c. The main target of the virus is T-cells lymphocytes
d. It is implicated in nasopharyngeal carcinoma

[Ref. Harrison 19/e, p 1188, 18/e, p 1467]

Primary target of EBV are B cells

18. Patient present in your clinic. On physical examination, there is bilateral lymphadenopthy, which is tender on palpation. He gave history of sexual contact. He is truck driver by profession. The probable causative agent is: **[MP 06]**

a. Herpes
b. LGV
c. H. ducreyi
d. Treponema

[Ref. Park 22/e 308; Harrison 18/e 1425]

Painful vesicles/ulcer single on multiple: *Herpes simplex*
Painless ulcer with shotty lymphnode: *Syphilis*
Painful ulcer with painful bubo: *Chancroid*
Painless ulcer with painful inguinal lymphadenopathy: *Lymphograncdoma venerum*

19. Which virus cannot be cultivated **[DNB 2013]**

a. Vaccinia
b. Variola
c. Molluscum contagous
d. Cowpox *[Ref. Ananthanarayan 9/e 465]*

20. African burkitt's lymphoma is cuased by: **[UP 06]**

a. Cytomegalo virus
b. EB virus
c. Herpes zoster
d. Infectious mononucleosis

[Ref. Harrison 19/e, p 1189, 18/e, p 1469]

21. Erythema infectiosum is caused by: **[Kar 06]**

a. Human parvovirus B-19
b. Papova virus
c. Human herpes virus type 8
d. Measles virus

[Ref. Ananthanarayan 9/e p 554]

22. Most common extra skin manifestation of varicella is involvement of: **[UP 07; SGPGI 05]**

a. CNS
b. Lungs
c. Kidneys
d. CVS

[Ref. Harrison 19/e, p 1184]

Most common extracutaneous site of involvement in V2V is CNS where the most common manifestation is acute cerebral ataxia and meningeal inflammation

23. Which one given below is a DNA virus? **[Comed 08]**

a. Poliovirus
b. Adenovirus
c. Parvovirus
d. Hepatitis-A virus

[Ref. Ananthanarayan 8/e, p 479, 9/e, p 481]

24. Adenovirus: **[UP 08]**

a. Double stranded DNA
b. Enveloped
c. Complex symmetry
d. None

[Ref. Ananthanarayan 8/e, p 479, 9/e, p 481]

25. Condyloma acuminatum is caused by **[DNB 2013]**

a. HSV
b. HPV
c. HIV
d. VZV

[Ref. Harrison 19/e, p 1198]

Anogenital wart are called as condylomata accumenata

26. Oral hairy leukoplakia caused by: **[APPG 08]**

a. Epstein barr virus
b. CMV
c. HIV
d. HZV

[Ref. Harrison 19/e, p 1188, 18/e, p 1469]

27. Adenovirus causes: **[Kerala 94]**

a. Keratoconjuctivitis
b. Diarrhoea
c. Parotid enlargement
d. All of the above

[Ref. Ananthanarayan 8/e, p 480, 9/e, p 481]

Answers 14. a. Herpes ... 15. c. Diagnosed ... 16. d. Leukemia 17. c. The main ... 18. b. LGV
19. c. Molluscum ... 20. b. EB virus 21. a. Human ... 22. a. CNS 23. b. Adenovirus
24. a. Double 25. b. HPV 26. a. Epstein 27. a and b

28. Adenovirus: [UP 08]
a. Double stranded DNA
b. Enveloped
c. Complex symmetry
d. None *[Ref. Ananthanarayan 8/e, p 479, 9/e, p 481]*

29. EB virus belongs to which group? [AI 95]
a. Retrovirus
b. Herpes virus
c. RNA virus
d. Pox virus

Note: Inverted papilloma is the MC neoplasm of nose and sinuses of unknown aetiology.

30. Herpes - zoster is caused by: [AI 99]
a. Herpes-simplex type I b. Herpes-simplex type II
c. Epstein-barr virus d. Varicella
[Ref. Ananthanarayan 8/e p. 481, 9/e p 472]

"Herpes zoster is reactivation of latent chickenpox virus (varicella zoster virus) from the dorsal root ganglia."

31. All of the following are true about Herpes group of virus except: [AI 98]
a. Ether-sensitive
b. May cause malignancy
c. HSV II involves below diaphragm
d. Burkitt's lymphoma involves T-cells
[Ref. Harrison 18/e p 1469; Greenwood 18/e, p 420]

Burkitt's lymphoma = ALL L3.
- ALL are tumors of relatively mature B cells.

32. Encephalitis is caused by: [AIIMS 98]
a. HSV-I b. EBV
c. Infectious mononucleosis d. CMV
[Ref. Greenwood 19/e, p 424]

33. Varicella are classified under: [AI 96]
a. Enterovirus b. Retrovirus
c. Poxvirus d. Herpes virus
[Ref. AA 8/e p 475, 9/e p 466]

34. Immunocompromised patient due to transplantation is suffering from pyrexia and neutropenia. Most likely cause is: [PGI 97]
a. HSV
b. CMV
c. Gram '-' ve organism
d. Gram '+' ve organism
[Ref. Harrison 18/e p 1123]

35. All of the following are true about the papovavirus except: [AI 95]
a. They are non-enveloped icosahedral viruses
b. Produce papilloma
c. RNA virus
d. SV-40 is oncogenic *[Ref. AA 8/e p 550, 9/e p 553]*

Papova virus are nonenveloped; Icosahedral human virus containing Ds DNA as genetic material.

Family Papova virus contains 2 genera:
1. Papilloma virus papilloma virus – Contains humans and animal
2. Polyoma virus – Contains SV-40 polyoma viruses

Answers 28. a. Double ... 29. b. Herpes virus 30. d. Varicella ... 31. d. Burkitt's ... 32. a. HSV-1
33. d. Herpes virus 34. b. CMVs 35. c. RNA ...

NEET Pattern Questions

1. **Lymphocytosis with atypical lympocytes are seen in infection with:** *[Ref. Harrison 19/e, p1187]*
   a. HSV b. HBV
   c. EBV d. RSV

2. **Paul Bunnell test is used for:**
   a. Malta fever
   b. Typhus fever
   c. Enteric fever
   d. Infectious mononucleosis
   [Ref. Ananthanarayan, 9/e, p 676]

3. **Paul bunnel reaction is a type of:**
   a. Agglutination b. CF
   c. Precipitation d. Flocculation test
   [Ref. Ananthanarayan, 9/e, p476]

4. **Human papillomatosis is caused by:**
   a. HSV b. HPV
   c. HIV d. HBV
   [Ref. Ananthanarayan, 9/e, p 553]

5. **HPV belongs to:** *[Ref. Ananthanarayan, 9/e, p 553]*
   a. Papova virus b. Parvovirus
   c. Herpes virus d. Poxvirus

6. **Flat warts is caused by which HP types:**
   a. 2, 4 b. 3, 10
   c. 16, 18 d. 5, 8
   [Ref. Ananthanarayan, 9/e, p 553; Harrison 18/e p1482; Greenwood 18/e p456]

Flat warts (verruca plana) are common among children and occur on the face, neck, chest and flexor surface of the forearm and legs. They are caused by: HPV 3 and HPV 10
Note:
Common wart: HPV 2, 4 and 7
Anogenital wart: HPV 6

7. **Most common type of HPV associated with cervical cancer:** *[Ref. Ananthanarayan, 9/e, p 553]*
   a. 6, 11 b. 5, 8
   c. 16, 18 d. 6, 8

8. **Condyloma accuminatum is caused by:**
   a. HSV b. HPV
   c. HIV d. VZV
   [Ref. Ananthanarayan, 9/e, p 553]

9. **HPV infects which cells first:**
   a. Superficial cells epidermis
   b. Basal cells
   c. Subcutaneous cells
   d. Dermal cells *[Ref. Harrison, 19/e, p 1197]*

Replication of HPV begins with infection of basal cells later on there is proliferation of all epidermal layers except the basal layer and produces acanthosis, parakeratosis and hyperkeratosis. Koilocytes appear in the granular layer.

10. **Most common Molluscum virus:**
    a. 1 b. 2
    c. 3 d. 4
    [Ref. Harrison, 18/e, p 1476]

11. **Congenital varicella infection causes all except:**
    a. Macrocephaly b. Limb hypoplasia
    c. Cortical atrophy d. Cicatrix
    [Ref. Harrison, 19/e, p 1184]

12. **Small pox belongs to which class of poxviruses:**
    a. Parapoxvirus b. Capripoxvirus
    c. Leporipox virus d. Orthopoxvirus
    [Ref. Harrison, 18/e, p 1477]

13. **Which pox wont grow in egg, animal cells:**
    a. Cow pox b. Vaccinia
    c. Variola d. Molluscum
    [Ref. Ananthanarayan, 9/e, p 465]

Molluscum virus cannot be grown in egg, tissue culture or animals.

14. **Shingles are seen in:** *[Ref. Harrison, 19/e, p 1184]*
    a. IMN b. Herpes zoster
    c. Chicken pox d. Small pox

15. **Following virus is a pox virus:**
    a. Variola b. Coxsachie
    c. ECHO d. HSV
    [Ref. Harrison, 19/e, p 220e]

Pox virus Genus
Orthopox virus: Variola, Monkey pox, cowpox, Buffalopox, vaccinia.
Molluscipox virus: Molluscum contagiousum
Parapox virus: Orf pseudocowpox, Deer pox, Seal pox
Yatapox virus: Tanapox

Answers **1. c.** EBV **2. d.** Infectious.... **3. a.** Agglutination **4. b.** HPV
5. a. Papova virus **6. b.** 3, 10 **7. c.** 16, 18 **8. b.** HPV
9. b. Basal cells **10. a.** 1 **11. a.** Macrocephaly **12. d.** Orthopox virus
13. d. Mollpscum **14. b.** Herpes zoster **15. a.** Variola

16. **HHV-8 causes:**
    a. Burkitt's lymphoma
    b. Nasopharyngeal carcinoma
    c. Kaposi sarcorna
    d. Hepatic carcinoma *[Ref. Harrison, 19/e, p 1194]*

17. **Slapped cheek sign is seen in:**
    a. Parvovirus B19
    b. JC virus
    c. Rota virus
    d. Mumps *[Ref. Greenwood, 18/e, p 478]*

18. **Pharyngoconjunctival fever is caused by:**
    a. Adenovirus 3 and 7
    b. Adenovirus 11, 21
    c. Adenovirus 40, 41
    d. Adenovirus 8, 10 *[Ref. Ananthanarayan, 9/e, p 482]*

19. **Adenovirus causes all except:**
    a. Hemorrhagic cystitis
    b. Diarrhea
    c. Respiratory tract infection
    d. IMN *[Ref. Ananthanarayan, 9/e, p 481]*

20. **18 years old girl presents with watery diarrhea. Most likely causative agent:**
    a. Rota virus
    b. V. cholerae
    c. Salmonella
    d. Shigella *[Ref. Ananthanarayan, 9/e, p 561]*

21. **Diagnosis of rotavirus is by:**
    a. Stool antigen
    b. Stool antibody
    c. Stool culture
    d. Blood antibody *[Ref. Ananthanarayan, 9/e, p 561]*

IgM and IgG antibodies can be demonstrated in the blood infected children.

22. **Brick-shaped virus:** *[Ref. Ananthanarayan, 9/e, p461]*
    a. Chicken pox
    b. Small pox
    c. CMV
    d. EBV

23. **HHV-6 causes:**
    a. Erythema infectiosum
    b. Kaposi sarcoma
    c. Roseola infantum
    d. Herpangina *[Ref. Ananthanarayan, 9/e, p 477]*

24. **Bivalent HPV vaccine contains which types:**
    a. Type 6, 11
    b. Type 6, 16
    c. Type 16, 18
    d. Type 11, 18 *[Ref. Harrison, 19/e, p 1199]*

HPV vaccine
Component: Virus like particles (VLPs) that consists of the HPV L_1 mayor capsid protein
- Bivalent vaccine: Contain HPV-16 and HPV-18
- Quadrivalent vaccine: HPV-6, 11, 16 and 18
- Second generation vaccine: HPV 16 and 18, additional oncogenic HPV 31, 33, 45, 52 and 58

25. **Cause of Herpes Zoster:**
    a. Primary infection with VZV
    b. Recurrent infection with VZV
    c. Reactivation of latent infection of VZV
    d. Multiple infection with VZV
    [Ref. Ananthanarayan, 9/e, p 472]

26. **Most common pox virus infection in human is:**
    a. Smallpox
    b. Monkeypox
    c. Cowpox
    d. Mulluscum contagiosum *[Ref. Harrison, 18/e, p 1476]*

27. **Most common cause of genital Herpes:**
    a. HSV-1
    b. HSV-2
    c. HSV-3 (VZV)
    d. EBV *[Ref. Ananthanarayan, 9/e, p 469]*

28. **Wrong statement about chicken pox/herpes zoster:**
    a. Caused by VZV
    b. Checken-pox primary infection
    c. Herpes-zoster recurrent infection
    d. Latent infection in trigeminal ganglion
    [Ref. Ananthanarayan, 9/e, p 473]

29. **CMV belongs to which family of DNA viruses:**
    a. Poxviridae
    b. Herpesviridae
    c. Papoaviridae
    d. Paravoviridae *[Ref. Ananthanarayan, 9/e, p 467]*

30. **Molluscum contagiosum virus belongs to:**
    a. Poxviruses
    b. Herpesviruses
    c. Picornaviruses
    d. Adenovirus *[Ref. Harrison, 19/e, p 1194]*

Answers 16. **c.** Kaposi sarcoma 17. **a.** Parvovirus B19 18. **a.** Adenovirus 3 and 7 19. **d.** IMN
20. **a.** Rota virus 21. **d.** Blood antibody 22. **b.** Small pox 23. **c.** Roseola infantum
24. **c.** Type 16, 18 25. **c.** Reactivation... 26. **d.** Mulluscum 27. **b.** HSV-2
28. **c.** Herpes-zoster... 29. **b.** Herpesviridae 30. **a.** Poxviruses

31. Suckling mice is used for isolation of:
a. Coxsachie virus
b. Pox
c. Herpes
d. Adenovirus *[Ref. Ananthanarayan, 9/e, p 434]*

Animal cultivation of virus
- Earliest method; Initially virus was inoculated into human volunteers
- White mice is the commonest animal used for this purpose
- Infant mice are very susceptible to cox-sackie and arbovirus
- Mice can be inoculated by several routes: intracerebral, subcutaneous, intraperitoneal or intranasal
- Disadvantage of animal inoculation are that immunity may interfere with viral growth and animal may horbour latent infection

32. True about CMV are all except:
a. Most common cause of post-transplantation infection
b. Most common cause of transplacental infection
c. A non-enveloped DNA virus
d. Produces intranuclear inclusions

All herpes virus are enveloped virus

33. Most common cause of sporadic encephalitis:
a. EBV b. HSV
c. Poliovirus d. CMV
[Ref. Harrison, 19/e, p 891]

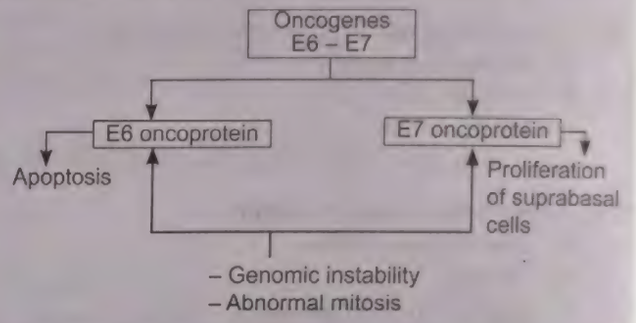
34. Least common cause of sporadic encephalitis:
a. HSV
b. VZV
c. Arbovirus
d. Rhinovirus *[Ref. Harrison, 19/e, p 891]*

35. Which of the following belongs to Herpesviridae:
a. Variola
b. Adenovirus
c. HPV
d. RK virus *[Ref. Ananthanarayan, 9/e, p 467]*

RK virus is the old name of HHV-7

36. Which viral gene acts as carcinogen in causing carcinoma cervix:
a. P24 -gene b. E -gene
c. L -gene d. H -gene
[Ref. Greenwood, 18/e, p 454]

HPV Genome
a. Early region:
– Two large frames E1 & E2
– Several small frames E4 – E7
b. Late region
– Two large genes
- E region encodes pathogenic proteins
- L region encodes regulator proteins
- E6 & E7 are the prime oncogenes which integrates with host chromosome (Note: Most HPV associated tumor therefore show integrated rather than episomal virus)



37. HPV causing cancer is:
a. HPV-6
b. HPV-11
c. HPV-16
d. HPV-3 *[Ref. Greenwood, 18/e, p 454]*

38. HPV causes which change in cervical epithelial:
a. Induction of apoptosis
b. Induction of necrosis
c. Immortalization of epithelial cells
d. None of the above *[Ref. Greenwood, 18/e, p 454]*

39. HPV-6 most often implicated in causation of:
a. Cervical cancer
b. Condyloma acuminata
c. Flat wart
d. Common wart *[Ref. Greenwood, 18/e, p 455]*

40. HPV causes which carcinoma:
a. Carcinoma cervix
b. Gastric carcinoma
c. Maxillary carcinoma
d. Colon carcinoma

Answers 31. a. Coxsachie virus 32. c. A non-enveloped... 33. b. HSV 34. d. Rhinovirus
35. d. RK virus 36. b. E -gene 37. c. HPV-16 38. c. Immortalization
39. b. Condyloma... 40. a. Carcinoma cervix

41. **Which is not a poxvirus:**
    a. Vaccinia virus
    b. Molluscum contagiosum
    c. Tanapox virus
    d. Coxsackie virus

42. **Oropharyngeal carcinoma is caused by:**
    a. HBV
    b. CMV
    c. HSV
    d. HPV
    [Ref. Harrison, 19/e, p 1197]

HPV is associated with invasive cancers of the anus, penis, vulva, vagina, cervix and a subset of oropharyngeal cancers.

43. **Low risk type of HPV:**
    a. Type-16
    b. Type-6
    c. Type-18
    d. Type-31

44. **Which of the herpes virus is included in Biohazard risk group 4:**
    a. HSV 1
    b. CMV
    c. EBV
    d. Herpes simiae

Biozarad Risk Group IV
It includes agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are not usually available.

45. **Vesicle in ear with facial nerve palsy on same side is due to which infection:**
    a. CMV
    b. VZV
    c. EBV
    d. HSV

46. **Castleman disease is associated with:**
    a. HSV
    b. CMV
    c. EBV
    d. HHV-8
    [Ref. Greenwood, 18/e, p 443]

47. **Which of the following is a wrong association:**
    a. HPV-CaCx
    b. EBV-Burkitt's lymphoma
    c. HHV 8-Kaposi sarcoma
    d. CMV-Nasopharyngeal carcinoma
    [Ref. Ananthanarayan, 9/e, p 473]

48. **Bollinger bodies are seen in:**
    a. Chickenpox
    b. Cowpox
    c. Fowlpox
    d. Smallpox
    [Ref. Ananthanarayan, 9/e, p 444]

Bollinger bodies are large inclusion bodies seen in fowl pox

49. **E6, E7 genes of which virus are implicated in oncogenesis:**
    a. EBV
    b. CMV
    c. HTLV-1
    d. HPV
    [Ref. Greenwood, 18/e, p 454]

50. **EBV enters B-cells through**
    a. CD-1
    b. CD-2
    c. CD-21
    d. CD-19
    [Ref. Ananthanarayan, 9/e, p 475]

51. **Human B-cell lymphotropic virus belongs to:**
    a. Picorna virus
    b. Pox virus
    c. Reovirus
    d. Herpes virus
    [Ref. Ananthanarayan, 9/e, p 477

Answers 41. d. Coxsackie virus 42. d. HPV 43. b. Type-6 44. c. EBV
45. b. VZV 46. d. HHV-8 47. d. CMV-Nasopharyngeal carcinoma
48. c. Fowlpox 49. d. HPV 50. c. CD-21 51. d. Herpes virus

CHAPTER

25

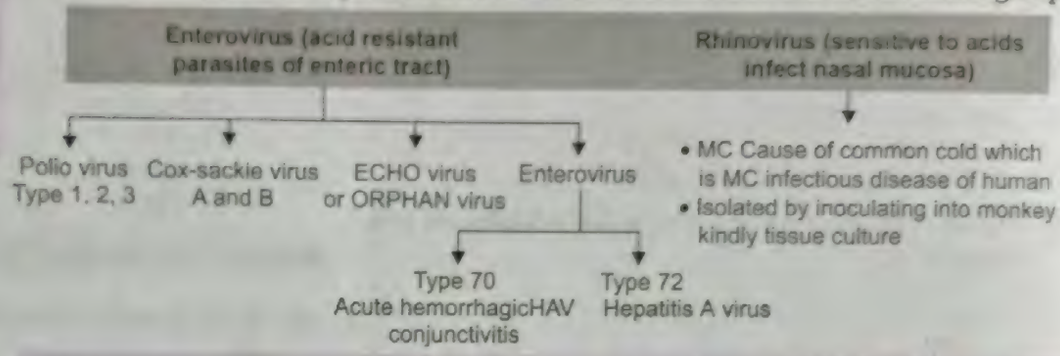
RNA Virus

PICORNAVIRUSES

I

Picornavirus
SS RNA virus family includes:
- Polio
- Coxsackie virus
- Echo virus
- Enterovirus

Icosahedral, nonenveloped SS RNA viruses, Inverse divided into two broad subgroups:



- Overall coxsackie virus B1 is the most common enterovirus infection followed by echovirus 18, 9 and 6. *... Harrison 18/e 1593*

Enterovirus lacks lipid envelope and are stable in acidic environment, including the stomach. They are susceptible to chlorine containing cleansers but resistant to inactivation by standard disinfectants (e.g. alcohol) and can persist for days at room temperature.

POLIO VIRUS

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Earliest neurogenic change of polio: Degeneration of Nissl bodies

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In March 2014 WHO announced the eradication of poliomyelitis from India.
The last case of polio in India was reported in 2011

- SS positive sense RNA virus, which does not survive lyophilization.
- *MC* type and most epidemics caused by **Type 1.**
- Epidemic caused by Type 1 and 3 while endemic is caused by Type 2.
- Vaccine induced paralysis is caused by mutated **Type 3.**
- Most **antigenic strain** - Type **2.**
- Two antigen – D or Native (N) antigen and C or heated (H) are identified.
- Anti D antibody is protective and used for measuring potency of injectable vaccine in terms of D antigen units.
- Transmitted by **feco-oral** route.
- **Earliest change** is the degeneration of Nissl bodies **(chromatolysis)** seen mostly in anterior horns of spinal cord (Signs of **lower motor neuron paralysis**).

Clinical Features

- I P 7–14 days. Manifest as:
  a. *Inapparent (subclinical) infection* - ***MC*** manifestation (95%).
  b. *Abortive polio or minor illness.*
  c. *Nonparalytic polio* - Mimics aseptic meningitis (~1%)
  d. *Paralytic polio* – Least common but severe manifestation (<1%)

Paralytic polio

- Can be biphasic (aseptic meningitis → recovery → fever/paralysis)
- Bulbar type paralysis causing dysphagia, difficulty in handling secretions or dysphonia
- Predominant sign - **Descending;** asymmetric; **proximal** more than distal; flaccid paralysis of **legs** *(MC)*, arms, abdominal, thoracic or bulbar muscles.

- **Objective sensory** testing usually yields **normal** results.
- *MC* muscle affected
- *MC* muscle which undergoes complete paralysis - *Quardriceps*
- *MC* muscle affected in hand - *Tibialis anterior*
- Common deformity at hip is flexion, abduction and external rotation. - *Opponens pollicis*
- **At knee** - flexion deformity is common but in severe cases *triple deformity* consists of flexion, posterior subluxation and external rotation occurs.
- Post polio syndrome is due to progressive dysfunction and loss of motor neurons that compensated for the lost neurons during original infection (not due to persistent/ reactivation of virus).
- **Risk of paralytic polio is increased by:**
  - Tonsillectomy
  - Adenoidectomy
  - Cortisone administration
  - Intramuscular injection
  - Strenous physical exercise
  - Pregnancy
  - Tooth extraction
  - Fatigue
  - Old age

I

Polio Virus
- Most epidemics are due to Type I
- Most antigenic: Type III
- Vacine induced paralysis: Type II
- MC muscle affected: Quadriceps femoris
- MC muscle undergos complete paralysis: Tibialis anterior

COXSACKIE VIRUS, ECHO VIRUS AND OTHER ENTEROVIRUSES

- *MC* clinical **manifestation of enterovirus** infection - Non-specific febrile illness *(Summer Grippe)* ...*Harrison 19/e, p 1291*
- *MC* cause of **aseptic** meningitis – *Enterovirus* (ECHO is *MC*)
- *MC* cause of **rubelliform** rash – *Echovirus 9*
- Transplacental Transmission occurs in coxsackie virus.

CLINICAL PRESENTATION OF ENTEROVIRUS

Manifestation	Coxsackie virus	Enterovirus	Echo
• Acute hemorrhagic conjunctivitis (characteristic subconjuctival hemorrhage)	A-24	Ent. 70	–
• Aseptic meningitis	Most group A, all B	Ent	E
• Encephalitis	A, B	Ent	E
• Exanthem	A, B	Ent	E
• Generalized disease of newborn	B_{2-5}	–	E
• Hand, foot and mouth disease	A, B (commonly by A)	Ent 71	–
• Herpangina	A, B (commonly by A)	Ent 71	–
• Myocarditis, pericarditis	A, B (commonly B)	–	–
• Paralysis	A, B (commonly A)	Ent	–
• Pleurodynia (Bornholm disease)	A, B (commonly B)	–	E
• Pneumonia	A, B	Ent	E
• Juvenile diabetes	B-4	–	–
• Orchitis	Coxasackie	–	–
• Post viral fatigue syndrome	B	–	–

I

It is necessary to employ suckling mice for the isolation of coxsackie virus

I

Among neonates enterovirus are the most common cause of aseptic meningitis and non-specific febrile illness

I

Echovirus 9 and 16 are frequently associated with fever and exanthen

Diagnosis of Enterovirus (Including Polio)

- *MC* **procedure** for diagnosis of infection - *Isolation* of enterovirus *in cell culture.*
- Isolation of virus from nasopharyngeal, throat sample, stool is sensitive but not specific (isolation from throat is more specific than from stool).
- Culture of CSF, serum, fluid from body cavities or tissues - less sensitive but specific.
- If CSF culture is negative than stool culture is done within first 2 weeks after onset of symptoms to confirm diagnosis.
- PCR of CSF - Highly sensitive and specific and rapid than culture.
- PCR of serum - Done for disseminated disease.
- Coxsackie virus may require inoculation into special cell-culture lines or into suckling mice.

I

- **MC** clinical manifestation of enterovirus infection: summer grippe
- **MC** enterovirus causing aseptic meningitis: Echovirus

Treatment

- IV/Intrathecal or intraventircular Ig for chronic enterovirus meningoencephalitis and dermatomyositis in patient with hypo or aggamaglobulinemia.
- *Pleconaril,* a drug once used is not available now.
- Glucocorticoids are contraindicated.

MYXOVIRUS

- Myxovirus is enveloped RNA virus, characterized by ability to adsorb onto mucoprotein receptors on erythrocytes causing hemagglutination.
- It is divided into two families - Orthomyxoviridae and Paramyxoviridae.

Distinguishing features of Orthomyxo and Paramyxovirus		
Features	**Orthomyxoviridae**	**Paramyxoviridae**
• *Genome*	Segmented (8 pieces)	Single linear RNA
• *Site of synthesis of Ribonucleo protein*	Nucleus	Cytoplasm
• *Genetic Reassortment*	Present	Absent
• *Antigenic Stability*	Variable	Stable
• *Hemolysis*	Absent	Present
• *Members*	Influenza virus	Measles (Morbillivirus), Mumps, Parainfluenza (Paramyxovirus), Respiratory syncytial virus (pneumovirus)

INFLUENZA

- Typically spherical virus divided into 3 subtypes (A, B, C) which are antigenically distinct.
- Type B and C occur almost exclusively in humans, whereas Influenza A exhibit a broad host range.

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Myxovirus
- Enveloped RNA virus family include orthomyxoviridae (influenzae) and paramyxoviridae (measles, mumps, parainfluenzae)
- Influenzae virus exhibit antigenic shift and antigenic drift
- H_1N_1 is the causative agent of most recent pandemic.

- **Antigenic structure**

  It has two types of antigens:

  a. Internal antigen: Type specific, i.e. (A, B, C) and stable.
  - Consists of RNP or soluble (S) antigen and M protein antigen.
  - It also includes envelope lipid antigen which is host specific.

  b. Surface/Viral or V antigen: Strain specific and show antigenic variations (A > B).

Antigenic variation is of 2 types:

(i) ***Major antigenic variation or antigenic shift***: It is due to *genetic recombination that is genetic reassortment* between animal and human virus and is **responsible for** *major epidemics or pandemic. Only* shown by type *A. Occurs every 10–15 years*

(ii) ***Minor antigenic variation or antigenic drift:*** It is due to *point mutation* and **is repsonsible for** *periodical epidemic.*
- Shown by type *A* and *B.*
- Type C does not show antigenic variation.

- Major pandemics are associated with antigenic shifts.

I
- B & C strains mainly seen in human
- A strain mainly infect water based wild birds.

- *V antigen* composed of at least 2 virus coded protein:

(i) ***Hemagglutinin (H):***
- Cause hemagglutination. Composed of two polypeptide HA_1 and HA_2.
- HA protein binds virus particle to susceptible cell and it is the major antigen against which neutralising antibodies are directed.

(ii) ***Neuraminidase (N)***
- Is receptor destroying enzyme (RDE) so cause elution.
- Anti-neuraminidase antibody is not as effective in protection as that of hemagglutinin.
- It facilitates release of virus particle from infected cell during budding.

- Viral subtypes are distinguished, according to their surface protein the hemagglutinin and neuraminidase (NA). *16* HA subtype and *9* NA subtype are found

- In world, three types of influenza virus are circulating - A (H_1N_1), A(H_3N_2), and B viruses.
- *New* influenza virus: A (H_5N_1), causative agent of bird flu.
- ***Source of Infection*** – Usually a case or subclinical.
- *Portal of entry* – Respiratory route
- *Incubation period* – 18-72 hours.
- Virus is readily killed by exposure to heat for 30 min at 56°C.
- Viruses are inactivated by detergents soaps, ethanol, halogens and phenolic compounds.

Note: WHO system of nomenclature of influenza A: Host of origin/geographical origin/strain No/year of isolation/antigenic description of hemagglutinin and neuraminidase example: swine/Iowa/3/70 (H_1N_1).

I

H5 N1 causative agent of avian influenza has GST postential of causing pandemic.

Clinical Features

- There is *no* viremia.
- Respiratory symptoms are prominent but abdominal pain and vomiting may occur in type B infections.
- *MC* complication is *pneumonia*. Mixed viral and bacterial pneumonia being most common.
- **Most serious** complication of influenza ***B virus*** is *Reye's syndrome [also occurs in influenza A and VZ virus].*
- Cardiac, neurological complication and gastric flu (with type B) may occur.
- ***MC* secondary** bacterial pneumonia in influenza - pneumococci. *Staphylococcus haemophilus.* ...*Harrison 19/e 1212*

Lab Diagnosis

- Virus isolation
  - **Best** specimen is *nasopharyngeal* secretion
  - Detected by indirect fluorescent antibody.
  - For primary isolation the most suitable cells are Madin-Darby canine kidney (MDCK) cells.

Prevention

1. **Best** is immunization. Vaccine is recommended only in certain selected population. Vaccine is of following types.
   a. ***Killed vaccine*:**
      Most commonly used vaccine:
      - *Contains H, N antigens*
      - *Usually one dose given but in patient with no previous immunological response 2 doses given*
      - *Immunity lasts for only 3-6 months.*
      - *Vaccine can produce very rarely Guillain-Barre syndrome (ascending paralysis).*

   b. ***Live attenuated vaccines (intra-nasal spray)*:**
      Administered as nose drops so induce both local and systemic immunity
   c. ***Newer vaccines*:**
      Split virus vaccine (sub-virion vaccine); Neuraminidase specific vaccine (sub-unit vaccine contains only N-antigen); Recombinant vaccine
2. **Antiviral drugs:**
   - For type A virus : Amantadine and rimantadine
   - For both A and B : Zamamivir, Oseltamivir.

Treatment

- Mainly symptomatic with acetaminophen. But NSAID should be avoided in pt < 18 years of age due to risk of Reye's syndrome.
- Maintain hydration and provide rest.
- **Specific antiviral:** Neuraminadase inhibitor oseltamivir and zanamivir for both type A & B Admantone agent amantadine and rimantidine for type A if sensitive.

I

Measles:
- RNA virus
- Exclusive human pathogen with very high secondary attack rate > 80%
- Warthin–finkeldey cells are pathognomic
- Koplik spot over buccal mucosa are pathognomonic exanthem

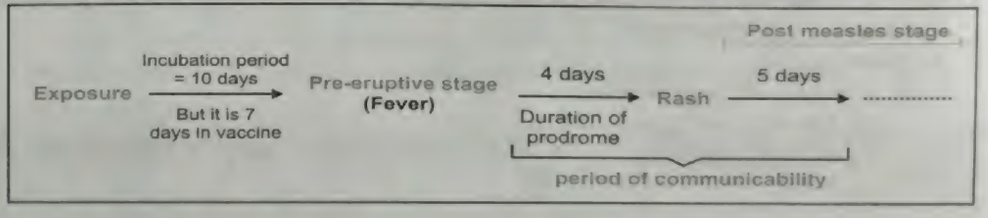
PARAMYXOVIRUS

- Family of enveloped viruses containing single strand of negative sense RNA.
- Resemble orthomyxovirus but are larger and more fragile.
- Enters the body through respiratory system.

Classification and important pathogens of the paramyxoviruses		
Genus	Human viruses	Animal viruses
Paramyxovirus	Paramyxovirus Parainfluenza viruses types 1, 3 Rubulavirus Mumps virus Parainfluenza viruses types 2, 4a, 4b	Newcastle diseases virus (NDV) (poultry), simian virus 5
Morbillivirus	Measles virus	Canine distemper virus, rinderpest virus, equine morbillivirus, morbilliviruses of seals, dolphins
Pneumovirus	Respiratory syncytial (RS) virus Human metapneumovirus (hMPV)	Turkey rhino-tracheitis virus (avian metapneumovirus)
Henipavirus	Hendra virus* Nipah virus*	Hendra virus Nipah virus
*These viruses cause disease in animals but can cause serious zoonotic human diseases		

MEASLES (RUBEOLA)

- A Morbillivirus
- RNA paramyxovirus, having *only one* serotype.
- *It cannot* survive outside the human body.
- *Carriers* are *not* known to occur. But subclinical cases occurs.
- Secondary attack rate > *80%*.
- Immunity after vaccination and infection is life-long.
- Multinucleated giant cells with inclusion bodies in the nucleus and cytoplasm ***(Warthin - Finkeldey Cells)*** in respiratory and lymphoid tissue are pathognomic for meases.
- Virion spike carry a haemagglutinin but not a neuraminidase function -F-protein is also a haemolysin.



Clinical Features

- ***Prodromal/Pre-eruptive stage***: A day or two before the appearance of rash **Koplik's spot** *(bluish white with erythematous halo)* appear on the buccal mucosa opposite the first and second upper molars.
  - It is **pathognomonic enanthem** of measles.
  - It disappears after the onset of rash.
  - Cough, coryza conjuctivitis and increasing fever is there.
- ***Eruptive stage:*** Rash appears on 4th day from fever. It begins at hairline and behind the ear and spreads downward rapidly. Characteristically it is erythematous, non-pruritic and maculopapular. Fever usually resolves by 4th or 5th day after the onset of rash. Prolong fever suggest complication.
- ***Postmeasles stage***

Remember: Modified measles occur in individual with pre-existing partial immunity induced by active or passive immunization.

I
- Measles during pregnancy is not associated with congenital abnormalities.
- However in pregnancy disease is more severe and can lead to abortion pre-term delivery.

....CMDT 2014 p. 1321

Diagnosis

- Best made by demonstration of measles specific IgM in blood or salivary sample.

Complication

- *MC* complications are - measles associated diarrhea, pneumonia and *otitis media* (*MC* complication **in young children**).
- More *serious* are neurological complication (febrile convulsions, encephalitis and sub-acute sclerosing pan encephalitis i.e. SSPE). Most cases result from immune mediated response to myelin proteins (post infectious encephalomyelitis) and not directly from viral infection.

Remember: Typical measles occur in person who received formalin inactivated measles vaccine in which rash begins peripherally and moves centrally.
Pneumonia is due to direct invasion of virus.

Prevention

1. Measles Vaccination:
   - *Live attenuated, tissue culture; freeze dried vaccine of HDC - Edmonston - Zagreb strain;* given to child as single subcutaneous dose of 0.5 ml as close to the age of 9 months.
   - Reconstituted in distilled water and should be used within one hour.
   - There is no spread of virus from vaccines to contacts.
   - Immunity develops in 11 to 12 days after vaccination.
   - One dose of vaccine give 95% protection.
   - Susceptible contacts may be protected by giving vaccine within 3 days of exposure.
   - Toxic shock syndrome occurs if vaccine is used after 4 hours of opening the vial.
   - Pregnancy is contraindication.
2. **Immunoglobulin**

Remember: Eradication is achieved when immunization coverage is at least 96%.

I
- Diarrhea. pneumonia and otitis media are the common complications of measles
- SSPE is the delayed complication of measles, related to prions.

MUMPS VIRUS = MYXOVIRUS PAROTIDITIS

- Paramyxovirus having predilection for glandular and nervous tissue.
- It has only one serotype.
- ***Maximum infectivity*** is just before and at the onset of parotitis.
- It spreads mainly by droplet infection.
- Humans are the only natural host. One attack (clinical or subclinical) induce life-long immunity.
- **Incubation Period –** Usually 14-18 days.

Clinical Features

- *Mumps* is the **most frequent** cause of *parotitis in children* in the age group 5-15 years.
- Usually bilateral parotitis occurs. Submandibular and sublingual gland can also be involved.
- Some patients develop pre-sternal edema.
- Other than parotitis, *orchitis* is the *MC* manifestation among *post- pubertal males*. May lead to testicular atrophy.
- *Aseptic meningitis* is a common manifestation in both children and adults.
- Glucose level in CSF may be abnormally low and this raises suspicion of bacterial meningitis.
- In pregnancy, it does not lead to premature birth or fetal malformation.

Diagnosis: Usually clinical

- RT DCR is the most sensitive test for detecting Virus in CSF
- For isolation monkey kidney or HEP2 cells are used.

Prevention

- Single dose of live-attenuated vaccine primarily in susceptible adults especially males who have not had mumps.
- ***Combined vaccine*** - MMR: At the age of 12-15 month and again at 4-6 years of age.

.... Harrison 18/e, p 1610

I

Mumps
- Exclusive human pathogen and is the most common cause of parotitis.
- After parotitis, orchitis is the most common manifestation.

PARAINFLUENZA VIRUS

- First parainfluenza virus to be discovered - Sendai virus or Hemagglutinating virus of Japan (HVJ) or influenza virus type D.
- It ranks second only to RSV as cause of lower respiratory tract illness.
- It has 4 types - 1, 2, 3, 4.

I

Parainfluenza virus:
- 2nd MC cause of lower respiratory tract illness
- Most serious manifestation is laryngotrachitis

Clinical Features

- **Most serious** clinical disease is *croup (laryngotracheobronchitis).*
- Para-influenza **type 1** is *MC* cause of croup in children.
- **Type 3** is important cause of lower respiratory disease (bronchitis, bronchiolitis and pneumonia) in infants.
- In older children and adults, most frequent symptom - common cold or hoarsenss.
- This virus confined to respiratory tract unlike mumps which is a systemic disease.

Diagnosis

- Isolation of virus from throat and nasal swabs by inoculating in primary monkey kidney cell cultures or continous monkey kidney cell line (LLC-MK2) with trypsin.

RESPIRATORY SYNCYTIAL VIRUS (RSV)

- An enveloped ssRNA virus.
- Most fragile among all paramyxovirus
- Lack haemagglutinin, hemolysin or neuraminidase.
- Broadly divided into two subgroups, Group A (mild disease) and Group B (severe)
- *MC* cause of *lower respiratory tract disease.*
- *MC* cause of *bronchiolitis seen* among infants between 1 and 6 months of age, peaking between 2 and 3 months of age.
- *MC* manifestation **in infants** rhinorrhea. Most serious manifestation is bronchiolitis.
- RSV is associated with sudden infant death syndrome
- **In adults** *MC* symptom are *common cold* with *rhinorrhea*, sore throat and cough.
- RSV is transmitted primarily by close contact with contaminated fingers or fomites.
- Incubation period is 4-6 days.
- Immunity is not long lasting.
- Nasal IgA is more protective than serum antibody.
- It produces fine rales; rhonchi, emphysematous change (that is hyperexpansion on chest X-ray) and atelectasis.

I

- ***Respiratory syncytial virus:***
  - SSRNA virus.
  - MC cause of lower RTI
  - MC cause of bronchiolitis

Diagnosis

- Specific diagnosis - by isolation of RSV from respiratory secretions (sputum, throat swab, nasopharyngeal wash).

Treatment

- ***Oxygen:*** Mainstay of therapy.
- ***Ribavirin:*** For infants who are severely ill or who are at high risk of complications of RSV infection (premature infants and those with bronchopulmonary dysplasia, congenital heart disease and immunosuppression).

HUMAN METAPNEUMOVIRUS

- Discovered in Netherlands in 2001 in bronchiolitis patients
- Closely resemble respiratory syncytial virus in all aspect
- It affects slightly older age group than RSV
- Diagnosis is made by RT-PCR
- Treatment is symptomatic

ROTAVIRUS AND OTHER VIRAL GASTROENTERITIS

- *MC* cause of diarrheal disease in infants and children: ***Rotaviurs***
- *MC* agent among older children and adults: Norovirus *(Norwalk like viruses)*

Table 227-1 from Harrison p No 1286.

ROTAVIRUS

- Non-enveloped, icosahedral virus with **segmented dsRNA (Character of Reovirus family)** so it exhibits *genetic reassortment.*
- VP-6 is major structural protein, which also is the target of commercial immunoassays and determines the group specificity of rotaviruses.
- There are seven major group. **Human illness** is caused *primarily* by **group A** and to a much lesser extent by group B and C.
- Adult diarrhea rota virus (ADRV) belongs to **group B** while **group C** causes pediatric gastroenteritis.
- 10 G serotypes of group A are identified in humans but 5 types ***(G1 through G4 and G9)*** are common.

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Rotavirus:
- MC cause of diarrhea in infant and children
- Non-enveloped segmental ds RNA virus
- Group A is the most common one
- Produce severe dehydration

Pathogenesis

1. It infects and destroys mature enterocytes in villous epithelum of proximal small intestine causing:
   a. Secretory diarrhea by ↓ villous epithelum + ↑ secretory crypt cells.
   b. Osmotic diarrhea – by ↓ brush border enzymes thereby accumulation of unmetabolized disaccharides.
2. Secretory diarrhea also results by:
   a. Enterotoxin (=NSP4) which alter epithelial cell function and permeability.
   b. Activation of enteric nervous system in the intestinal wall leads to increased fluid secretion.

...Harrison 19/e, p 1288, 18/e, p 1591

I

Rotavirus produce secretory diarrhea by ↓ villous epithelium

Clinical Features

- *Incubation period:* 1-3 days.
- It infects all children by 3-5 years. May also infect neonates.
- Peak age - 4 to 23 months.
- Occurs predominantly during the winter months.
- Transmitted predominantly through feco-oral route. Also transmitted by respiratory secretion, person to person, contaminated environmental surface.
- ***Severity of dehydration:*** Rotavirus > Norovirus > Sapovirus
- ***Vomiting*** frequently ***precedes*** the diarrhea.
- Stools are characteristically loose and watery and only infrequently contain red or white cells.
- Rotavirus is associated with respiratory and neurologic features, sudden infant death syndrome, necrotizing enterocolitis, intussusception and diabetes mellitus type I.
- Its infection produces virus specific secretory IgA in intestine and IgA, IgM, IgG in serum which increase with each reinfection, so severe disease is ***More common*** in young children with first or second infections.

Diagnosis

- As virus is shed in large quantities (10^7–10^{12}/g) in stool, the diagnosis is ***confirmed by*** detecting viral antigen in feces by Enzyme immunoassays and by detecting viral RNA (by gel electrophoresis, probe hybridization or PCR).
- Human rotavirus does ***not grow*** readily in cell culture.

Treatment

- Rehydration therapy is given.
- Antibiotics and antimotility agents avoided.
- In immunocompromised children - oral immunoglobulin or colostrum given.

Prevention

- First licensed rotavirus vaccine (Rotashield) was withdrawn due to its association with intussusception.
- Two new live attenuated oral vaccine have been introduced:
  a. Pentavalent bovine human reassortant rotavirus vaccine (RotaTeq) contains G_1, G_2, G_3, G_4 and P(8).
  b. Single attenuated human rotavirus strain (Rotarix) contains P_1 A(8)G_1

OTHER VIRAL GASTROENTERITIS

1. **Norwalk and Related Human Calciviruses:**
   - Small rounded icosahedral ssRNA virus, broadly classified into two genera: *Norovirus* (previously called Norwalk like virus) and *sapovirus* (previously called sapporos like virus).
   - Norovirus is the ***MC*** infectious agent of mild gastroenteritis in the community and affects all age groups whereas sapoviruses primarily cause gastroenteritis in children.
   - Noroviruses are the major cause of epidemics of gastroenteritis worldwide.

   Pathogenesis:
   - It gets attached on carbohydrates (similar to human histoblood group antigens) of duodenal epithelium of individuals with the secretor phenotype (genetic predisposition to illness).
   - Reversible lesion in upper jejunum, e.g. broadening and blunting of villi, shortening of microvilli, etc.
   - Malabsorption of carbohydrates and fats and decreased brush border enzymes.
   - Adenylate cyclase activity is not altered.
   - Gastric motor function is delayed but histological changes are absent in stomach and colon.

   Modes of Transmission:
   - Transmission occurs predominantly by fecal-oral route but virus is also present in vomitus.
   - Also transmitted by aerosolization, contact with contaminated fomites, person to person contact.
   - Shellfish harvested from fecally contaminated water pose a special risk.

   Clinical Features:
   - ***Vomiting*** is more common among children where adults usually develop ***diarrhea.***
   - Constitutional symptoms like fever, chills and rigor are common.
   - ***Stools*** are characteristically loose and watery without blood, mucus or leukocytes.
   - There is paradoxic inverse association between level of antibody and protection from disease that is person with higher level of pre-existing antibody are more susceptible to illness.

   Diagnosis:
   - PCR for detection of virus in stool and vomitus.
   - EIA (Enzyme immunoassays) for detection of virus in stool and serologic response to specific viral antigen.
   - It has not yet been propagated in cell cultures.

   Treatment:
   - Generally not required since it is self-limited.

2. **Adenovirus:** – Enteric adenovirus (40 and 41) are difficult to cultivate in cell lines.
3. **Astrovirus:** – Serotype 1 is ***MC.***
4. **Torovirus:** – Cause less vomiting and more bloody diarrhea.
5. **Picobirnaviruses:** – Bi-segmented ***double stranded RNA*** virus.
   – Cause gastroenteritis ***in HIV*** infected adults.
6. **Hendra and Nepah viruses:** – Classified under paramyxoviridae family. Cause gastroenteritis in persons in contact with pigs.
7. **SARS-Cov:** (Severe acute respiratory syndrome associated coronavirus).
8. **Enteroviruses, reoviruses, pestiviruses, parvovirus B.**

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Norovirus is the MC cause of mild gastroenteritis
Adenovirus, Astrovirus, Torovirus are other enterovirus

ARBOVIRUSES = ARTHOPOD BORNE VIRUSES

- The most important arbovirus vectors are mosquitoes followed by ticks.
- Most arbovirus agglutinate red cells (Hemagglutination) but spontaneous elution does not occur.
- Arboviruses have been placed in Toga, Flavi, Bunya, Reo and Rhabdovirus families.
- Arboviruses known to be prevalent in India are:

Group A	Group B	Others	
(Alphaviruses)	(Flaviviruses)	Umbre	Chandipura
Sindbis	Dengue	Sathuperi	Chittoor
Chikungunya	Kyasanur Forest disease	African horse sickness	Minnal
	Japanese encephalitis	Venkat puram	Sandfly fever
	West Nile	Kaisodi	Vellore

- *Clinical syndromes of Arboviues:*
  - **Febrile group:**
    - *MC* group
    - No rash and arthralgia seen
      e.g. Sindbis, Chikungunya, dengue (Types 1-4), Westnile, Sandfly fever, Rift valley fever.
  - **Hemorrhagic fevers (HF):**
    - Dengue, chikungunya, kyasanur forest disease, lassa fever, yellow fever, marburg or ebola HF, hantavirus pulmonary syndrome, HF with renal syndrome, rift valley fever, crimean congo HF, omsk HF.
  - **Encephalitis:**
    - West Nile, Japanese encephalitis.

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- Most arboviral disease are transmitted by mosquito:
- Chikungunya is a abroviral disease related to alpha viruses

RHABDOVIRIDAE

- **Bullet shapted**, enveloped viruses with SS RNA genome are known as Rhabdovirus.
- Rhabdoviridae contain two genera:
  (i) Vesiculovirus containing vesicular stomatitis virus, chandipura virus.
  (ii) Lyssavirus containing Rabies virus, Lagos bat, Mokola, Duvenhage.

RABIES VIRUS (LYSSAVIRUS SEROTYPE 1)

- Unsegmented, linear negative, **neurotropic**, RNA virus which causes direct zoonosis of **warm blooded animals** (particularly carnivorous such as dogs, cats, jackals and wolves) including man called as RABIES.
- Serotype 2, 3, 4 are *rabies related virus.*
- Viral genome encodes for five proteins: nucleocapsid, matrix, phosphoprotein, glycoprotein and RNA polymerase.
- It has two major antigens – glycoprotein (G Protein) and nucleocaspid protein.
- Glycoprotein seems to be the only antigen capable of inducing the formation of virus neutralizing (protective) and hemagglutination - inhibiting antibodies.
- Virus excreted in the saliva of rabid animals is called 'street virus' which is pathogenic for all mammals and has long **variable** incubation period.
- ***Serial brain to brain passage of Street Virus modifies it into fixed virus which has following characteristics:***
  - Short, fixed and reproducible incubation period.
  - Not form Negri bodies and not multiply in extraneural tissues.
  - Used in the preparation of anti-rabies vaccine.
  - It is pathogenic under certain conditions, e.g. when inadequately inactivated for vaccine production.
- Rabies is only communicable disease of man that is *always fatal.*

Remember: Rabies is dead end infection.

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Rabies:
- Bullet shaped SS RNA virus
- Maldive is the only country where there is no rabies.
- Virus first spread centripetally and then centrifugally.
- Negri bodies are pathognomonic

Type of Rabies

(i) ***Urban rabies:*** Caused by the dog and is responsible for 99% of human cases in India.
(ii) Sylvatic or wild-life rabies.
(iii) Bat rabies.

Remember:
- In most of the world, dog is the most important vector.
- Maldives is the only country which does not have human or animal rabies.
- In India, rabies occur in all parts except Lakshadweep and Andman and Nicobar Islands
- Most effective natural barrier to rabies - water.

Mode of transmission:
- *Animal bites* - ***MC***
- Licks on abraded skin and abraded or unabraded mucosa.
- Respiratory (aerosol) transmission.
- Person to person - rare
- Also by corneal and organ transplants.

Incubation period: Highly variable depending on the site of bite (i.e. actual distance that the virus has to travel to reach to CNS), severity of bite, etc. It is usually 1-3 months but can be < 2 week or > 1 year.

Clinical Features

- Virus spreads ***centripetally*** from site of infection (striated muscle) then ascends through nerve associated tissue space, and then spreads ***centrifugally*** in peripheral autonomic nerves to many tissues.
- Once the virus enters the CNS, it rapidly disseminates to other regions of the CNS via fast axonal transport along neuro-anatomic connections.
- Rabies ***prominantly infect neurons***, infection of astrocytes is unusual.
- After establishing CNS infection, there is centrifugal spread to other tissues including salivary gland, heart, adrenal gland and skin.
- Salivary gland invasion is ***crucial*** for transmission of virus.
- Pathological study shows mild inflammatory changes in the CNS with mononuclear cells in leptomeninges, perivascular regions and parenchyma including microglial nodules called ***Babes nodules*** *...Harrison 19/e, p1301*
- Most characteristic pathologic finding in CNS is the formation of cytoplasmic inclusions called ***Negri Bodies*** *(composed of finely fibrillar matrix and rabies virus particles)* within neurons of Ammon's horn, cerebral cortex, brain stem, hypothalamus, purkinje cells of the cerebellum and dorsal spinal ganglia. However, Negri bodies are not observed in all cases of rabies.
- It has four stages:
  a. *Prodromal period*: Specific symptom is complaint of paresthesia/fasciculation at or around the site of inoculation of virus, fever, headache, lethargy are common constitutional symptoms.
  b. *Encephalitic phase*: Abnormalities of automatic nervous system. Aerophobia (pathognomic) and Hydrophobia (pathognomic and absent in animals) may be seen, hypersalivation, aphasia.
  c. ***Manifestation of brain stem dysfunction***: The prominence of early brainstem dysfunction distinguishes it from other viral encephalitis.
  d. *Coma and Death.*
  e. *Paralytic Rabies*: In about 20% of patients cardinal features of encephalitic rabies are lacking. Rather they exhibit early and prominant flaccid paralysis of involved extremity that progress to quadriparesis and death.

Remember: It may be also present as ascending paralysis resembling GBS, most frequently among persons given post-exposure prophylaxis after being bitten by vampire bats

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- Negri bodies are made up of fibrillar matrix and rabies viral particles
- Cell culture vaccine are derived from fixed rabies virus

Diagnosis

- Confirmed by antigen detection using immunofluorescence of infected tissue (corneal impression smear, skin biopsy or brain) and by virus isolation from saliva and other secretions.
- The commonly performed direct fluorescent antibody testing of skin biopsy material from posterior neck (where hair follicles are highly innervated) has a sensitivity of 60-80%.

.....CMDT 2014, p 1320

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Rabies virus spread centripetally towards the CNS at a rate of 250 mm/d Via retrograde fast axonal transport to the spinal cord or brainstem.

Prevention Types of Vaccine

Rabies can never be cured but can always be prevented with passive + active immunization. There are various vaccine available:

(a) Nervous tissue vaccine (NTV)
- From suckling mouse brain
- From adult animal tissues (e.g. sheep): Semple type

(b) Duck embryo vaccine (DEV)
- Not available in India

(c) Cell culture vaccine

CELL CULTURE VACCINE	
Human diploid cell (HDC) vaccine: In India it is used for both pre and post exposure prophylaxis (PEP). It is a purified preparation of fixed rabies virus grown on human diploid cell.	***"Second generation" tissue culture (animal cell) vaccines,*** i.e. of non human origin, e.g. chick embryo fibroblast, vero cells. The WHO recommended that culture of HDC line should be replaced by culture of animal cell line

Types of Prophylaxis

1. *Post-exposure prophylaxis*: Combined administration of single dose of antirabies serum with a course of vaccine, and local treatment of wound is the *best* specific prophylactic treatment after exposure of man to rabies.

   Indication of Anti-rabies Treatment:
   a. If animal shows sign of rabies within 10 days
   b. Biting animal can't be traced
   c. Unprovoked bites
   d. Laboratory test (fluorescent rabies antibody test or test for negri bodies) of brain of biting animal are positive.
   e. All bites by wild animals.

Classification of exposures		
Class I (slight risk)	**Class II (Moderate risk)**	**Class III (severe risk)**
• Licks on healthy unbroken skin	• Licks on fresh cuts	• All bites or scratches with oozing of blood on neck, head, face, palm, fingers
• Consumption of unboiled milk of suspected animal	• Scratches with oozing of blood	• Lacerated wounds on any part of body
• Scratches without oozing of blood	• All bites except on head, neck, face, palm, fingers	• Multiple wounds 5 or more in numbers
	• Minor wounds less than 5 in number	• Bites from wild animals

- The optimal form of passive immunisation is human rabies immunoglobulin (20 IU/kg) administered once, the full dose should be infiltrated around the wound. Any remaining amount can be infected intramuscularly at a site distant from wound.
- Along with this active immunization by vaccine (HDCV or purified chick embro cell vaccine) must be administered as per **standard WHO intramuscular regimen:** 0, 3, 7, 14, 28, days and booster on 90 days.

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Class III dog bite are most risky, it includes bite from wild animals.

2. *Preexposure prophylaxis*
   - Given to laboratory staff working with rabies virus, veterinarians, etc.
   - Cell culture vaccines on 0, 7, 28 days.
   - If titer of neutralizing antibody in serum taken after 1 month of 3rd dose, is less than 0.5 IU/ml then administer booster until antibodies become demonstrable.
3. *Postexposure treatment of persons who have been vaccinated previously:*
   - If titer of antibody > 0.5 IU/ml and bite is not severe – 2 doses (days 0, 3).
   - If titer of antibody is unknown or bite is severe - 3 doses of HDC on 0, 3, 7 days.

Multiple Choice Questions

Polio and Enterovirus

1. **All of the following statements are true regarding poliovirus except:** [AI 04]
   a. It is transmitted by feco-oral route
   b. Asymptomatic infections are common in children
   c. There is a single serotype causing infection
   d. Live attenuated vaccine produces herd immunity

2. **Which of the following is the most common cause of meningoencephalitis in children:** [AI 2011]
   a. Mumps
   b. Arbovirus
   c. HSV
   d. Enterovirus

3. **All of the following clinical features are associated with enteroviruses except:** [AI 04]
   a. Mycocarditis
   b. Pleurodynia
   c. Herpangina
   d. Hemorrhagic fever

4. **True about polio:** [AIIMS 08]
   a. Paralytic polio is most common
   b. Only one type exists
   c. Increased muscular activity leads to increased paralysis
   d. Polio drop given only in <3 years

5. **All are false regarding polio virus except:**
   a. Most cases are symptomatic [AIIMS Nov 07]
   b. Inactivated vaccine given IM
   c. Inactivated polio vaccines are given to child less than 3 years of age
   d. Only one type exists

6. **All are true about poliovirus except:** [AIIMS 02]
   a. Type 1 is responsible for most epidemics
   b. Very difficult to eliminate type 1
   c. Type I is responsible for vaccine induced paralytic poliomyelitis
   d. Type I most commonly associated with paralysis

7. **Enterovirus causes all except:** [AIIMS 01]
   a. Hemorrhagic fever
   b. Pleurodynia
   c. Herpangina
   d. Aseptic meningitis

Influenza and Parainfluenza

8 (a). **True Statement about Enteroviruses:** [PGI May 2013]
   a. Composed of segmented RNA genome
   b. Stable at pH 4
   c. Cause pleurodynia
   d. Cause encephalitis
   e. Cause meningitis

8 (b). **70-year-old woman refused to take influenza vaccine, devloped flu. Death happened 1 week after pneumonia. Causes of post influenza pneumonia:** [AIIMS Nov 2014]
   a. Staphylococcus
   b. Measles
   c. Ligionella
   d. CMV

9. **H_5N_1 is:** [AI 08]
   a. Bird flu virus
   b. Vaccine for HIV
   c. Causative agent of Japanese encpehalitis
   d. An eradicated virus

10. **True statement about influenza A virus:** [AI 04]
    a. It has a double stranded segmented RNA
    b. Pandemics are caused by antigenic drifts
    c. Nucelocapsid antibody is not specific
    d. Hemagglutinin and neuraminidase are strain specific

11. **Segmented RNA is found in:** [AIIMS 97, PGI 00]
    a. Influenza virus
    b. Rabies virus
    c. Herpes virus
    d. Molluscum contagiosum virus

12. **Incubation period less than 10 days seen in:** [PGI 02]
    a. Influenza
    b. Cholera
    c. Plague
    d. Chickenpox
    e. Rabies

Measles and Mumps

13. **Which of the following is not true about measles?**
    a. High secondary attack [AI 08]
    b. Only one strain causes infection
    c. Not infectious in prodromal period
    d. Infections confer life-long immunity

14. **With reference to mumps which of the following is true?** [AI 06]
    a. Meningoencephalitis can precede parotitis
    b. Salivary gland involvement is limited to the parotids
    c. The patient is not infectious prior to clinical parotid enlargement
    d. Mumps orchitis frequently leads to infertility

15. **Commonest complication of Mumps is:** [AI 00]
    a. Orchitis and Oophoritis
    b. Encephalitis
    c. Pneumonia
    d. Myocarditis

16. **Regarding mumps, which is true?** [AIIMS Nov 07]
    a. Causes SSPE
    b. Mumps causes aseptic meningitis in children
    c. Sublingual gland is involved commonly
    d. All

17. **Which of the following is the 'Least common' complication of measles ?** [AIIMS 06]
a. Diarrhea b. Pneumonia
c. Otitis media d. SSPE

Rotavirus & Norovirus

18a. **A patient comes to your clinic with a complaint of multiple episodes of loose watery stool for 3 days. On probing, you discover that these episodes start after he had ingested shellfish at a local restaurant 3 days back and other people who had food from that restaurant had similar symptoms. What is the most likely cause of viral diarrhea in these adults?:** [AIIMS 15]
a. Adenovirus b. Calcivirus
c. Rotovirus d. Norovirus

18b. **Rotavirus is detected by:** [AIIMS 02]
a. Antigen in stool
b. Antibody in serum
c. Demonstration of virus
d. Stool culture

19. **Vaccination causing intussuception:** [PGI Dec. 07]
a. Rotavirus b. Parvovirus
c. Inactivated polio d. BCG
e. Measles

20. **Reassortment is typically seen in:** [AIIMS Nov 10]
a. Herpes b. Hepadna
c. Rotavirus d. Astrovirus

21. **Vaccines prepared by embryonated hen's egg are:**
a. Measles b. Rabies [PGI 04]
c. Rubella d. Varicella

Rabies

22. **Negro body is seen in:** [AI 07]
a. CMV
b. Rabies
c. Inclusion of herpes simplex
d. EBV

23. **For the treatment of case of class III dog bite, all of the following are correct except:** [AI 05]
a. Give Ig for passive immunity
b. Give ARV
c. Immediately stitch wound under antibiotic coverage
d. Immediately wash wound with soap and water

24. **Class II exposure in animal bites includes the following:**
a. Scratches without oozing of blood [AI 03]
b. Licks on a fresh wound
c. Scratch with oozing of blood on palm
d. Bites from wild animals

25. **Which of the following statements is true about rabies virus?** [AI 03]
a. It is double stranded - RNA virus
b. Contains a DNA-dependent RNA polymerase
c. RNA has a negative polarity
d. Affects motor neurons

26. **Regarding rabies, true is:** [AI 00]
a. Incubation period depends on the site of bite
b. Diagnosis is by eosinophilic intranuclear inclusion c. It is a DNA virus
d. Caused only by dogs

27. **A 25-year-old girl has admitted to hospital with provisional diagnosis of rabies. The most suitable clinical sample that can confirm the antemortem diagnosis is:**
a. Serum for antivirus IgG antibody [AIIMS 04]
b. Corneal impression smear for immuno-fluorescence stain
c. CSF sample for viral culture
d. Giemsa stain on smear prepared from salivary secretions

28(i). **A boy got unprovoked bite from a neighbour's dog. The animal control authority caught the dog and it was found to be healthy. What will be the next step?** [AIIMS May 10, AI 2010]
a. Test antibody level in the dog
b. Withhold immunization and observe the dog for 10 days for signs of rabies
c. Start post-exposure prophylaxis
d. Perform euthanasia for the dog

28(ii). **Diagnostic of Rabies:** [AIIMS May 10, AI 2010]
a. Guarneri bodies b. Negri bodies
c. Cowdry Aody d. Bollinger bodies

28(iii). **A patient presented to the hospital with severe hydrophobia. You suspect rabies obtain corneal scrapings from the patient. What test should be done on this specimen for a diagnosis of Rabies?**
a. RT-PCR for rabies virus [AIIMS Nov 2016]
b. Negri bodies
c. Antibodies to rabies virus
d. Tzanck smear

Arbovirus

29. **Soft tick transmits:** [AI 08]
a. Relapsing fever b. KFD
c. Tick typhus d. Tularemia

30. **Which of the following viral infections is transmitted by tick?** [AI 05]
a. Japanese encephalitis
b. Dengue fever
c. Kyasanur forest disease (KFD)
d. Yellow fever

31. **Which is true about arboviral disease?** [AI 00]
a. Yellow fever is endemic in India
b. Dengue viruses have only one serotype
c. KFD is transmitted by ticks
d. Japanese encephalitis is transmitted by Aedes

32. **Mark true in following:** [AIIMS 08]
a. Hanta virus pulmonary syndrome is caused by inhalation of rodent urine and feces
b. Kyasanur forest disese is caused by bite of wild animal
c. Lyssa virus is transmitted by ticks
d. Chikugunya is caused by anopheles

33. **Following are arboviral disease:** [PGI 03]
    a. KFD
    b. West Nile Fever
    c. Ganjam virus
    d. RSV
    e. Puumala virus

34. **In Japanese encephalitis pigs acts as:** [PGI 00]
    a. Amplifier
    b. Definitive host
    c. Intermediate host
    d. Any of the above

35. **Most specific for Dengue diagnosis** [AIIMS 08]
    a. IgM ELISA
    b. Tissue culture
    c. CFT
    d. Electron microscopy

36. **Which of the following is/are arboviral diseases?**
    a. Japanese encephalitis [PGI June 09]
    b. Dengue
    c. Yellow fever
    d. Hand-foot-mouth disease
    e. Rocky mountain spotted fever

37a. **True about dengue fever** [PGI May 2013]
    a. Caused by 4 serotypes
    b. Effective vaccine is available
    c. Presents with fever and joint pain
    d. Virus belongs to flavivirus genus
    e. Contain segmented RNA

37b. **In a suspected patient of dengue, all of these are acceptable investigations at day 3 of presentation except:**
    a. NS1 antigen detection [AIIMS Nov 2016]
    b. Viral culture and isolation in C6/36 cell line
    c. RT-PCR
    d. ELISA for antibody against dengre virus

Other

38. **The most common etiological agent for acute bronchiolitis in infancy is:** [AI 06]
    a. Influenza virus
    b. Parainfluenza virus
    c. Rhinovirus
    d. Respiratory syncytial virus

39. **All of the following statements are true about congenital rubella except:** [AI 05]
    a. It is diagnosed when the infant has IgM antibodies at birth
    b. It is diagnosed when IgG antibodies persist for more than 6 months
    c. MC congenital defects are deafness, cardiac malformation and cataract
    d. Infection after 16 weeks of gestation result in major congenital defects

40. **Laboratory diagnosis of viral respiratory tract infections can be established by all of the following tests except:** [AI 04]
    a. Detection of virus specific IgM antibodies in single serum specimen
    b. Demonstration of viral antigens by indirect immunofluorescence assay in nasopharyngeal washings
    c. Isolation of viruses using centrifugation enhanced culture
    d. Detection of viral hemagglutination inhibiting (HAI) antibodies in a single serum specimen

41. **Risk of the damage of fetus by maternal rubella is maximum if mother gets infected during:** [AIIMS 05]
    a. 6-12 weeks of pregnancy
    b. 20-24 weeks of pregnancy
    c. 24-28 weeks of pregnancy
    d. 32-36 weeks of pregnancy

42. **A 11-month-old child presents with complaints of respiratory distress. On examination there is bilateral crepitation and wheezing. Which of the following is the most likely cause?** [AIIMS 00]
    a. Pneumonia
    b. Adenovirus
    c. Respiratory syncytial virus
    d. Rhinovirus

43. **All are included in picorna group of viruses except:**
    a. Encephalo myocarditis
    b. HEV [PGI 04]
    c. Foot and mouth virus
    d. Polio virus

44. **Choose the correct matches:** [PGI 02]
    a. Mumps-RA 27/3 strain
    b. Rubella-Jeryl-Lynn strain
    c. Measles-Edmonston-Zagreb strain
    d. BCG-Danish 1331 strain

45. **Micro-organism used as weapon in biological terrorism:**
    a. Small pox virus
    b. Rabies virus [PGI 02]
    c. Ebola virus
    d. Influenza C virus
    e. Human parvovirus

46. **Organism (s) causing, bronchiolitis in infant:** [PGI 00]
    a. RSV
    b. Rhinovirus
    c. Parainfluenza
    d. Influenza
    e. *H. influenzae*

47. **All are RNA virus except:** [AIIMS 08]
    a. Ebola virus
    b. Vesicular stomatitis virus
    c. Simian 40
    d. Rabies

48. **All are true regarding hantaan virus except:**
    a. DNA virus [PGI 2005, AIIMS 96]
    b. Carried by rodents
    c. Causes recurrent respiratory infection
    d. Hemorrhagic manifestation may occur

49. **New infectious agents are:** [PGI 07]
    a. Nipah virus
    b. Pneumocystis jeruveci
    c. Corona virus
    d. SARS
    e. Prion

50. **True about hanta virus:** [PGI 02]
    a. Hantavirus pulmonary syndrome
    b. Transmitted by arthropod
    c. Transmitted by rodents
    d. Hemorrhagic fever with renal failure
    e. Hantavirus pulmonary symdrome acquired from person to person

51. **True about Cirmean-Congo haemorrhagic fever:**
    a. Zoonosis [PGI Nov 11]
    b. Develop petechial rashes
    c. Transmitted by mites
    d. Recently disease has been reported in Gujarat
    e. It has high fatality

Explanations and References with Illustrative Answers

1. **Ans. (c) There is a single serotype causing infection** *Ref. Park 22/e, p 184-186; Ananthanarayan 8e, p 484, 9/e, p 485-486*

Important features of Polio virus

- Poliovirus, causative agent of polio, is SS positive sense RNA virus having 1, 2 and 3 serotypes.
- *MC* serotype - *Type 1*
- *MC* cause of epidemic - Type 1 (epidemics are also caused by Type 3 while endemics are by Type 2)
- *MC* type associated with paralysis - *Type 1*
- Most difficult to eradicate Type -1
- Most antigenic - Type 2
- *MC* cause of vaccine induced paralysis - ***mutated Type - 3***
- **Modes of transmission**
  - *Main route*: Feco-oral route directly or indirectly.
  - *Droplet infection*: May occur in acute phase of disease.
- **Clinical Spectrum**
  - Most vulnerable age is between 6 months and 3 years.
  - M.C clinical presentation – Inapparent / subclinical infection.
  - Most rare clinical presentation – Paralytic polio.
  - Usual cause of death – respiratory insufficiency.
  - Progressive paralysis, coma or convulsions usually indicate cause other than polio.
- **Prevention**
  - Two types of vaccine:
    - i. *Inactivated (salk) injectable polio vaccine.*
    - ii. *Oral (sabin) live attenuated vaccine:*
      - It provides both local immunity (by producing intestinal IgA) and systemic immunity.
      - Vaccine progeny is excreted in feces and secondary spread occurs to household contacts so non immunized persons are immunized by replacing wild strain by vaccine strain.
      - It results in herd immunity even if only about 66% of community is immunized.

Mnemonics: = For Live Attenuated Vaccine (TIPS BYE C_2MMR)

T	I	P	S
Typhoid oral	Influenza	Plague	Sabin
B	Y	E	
BCG	Yellow Fever	Epidemic typhus	
C2	M	M	R
Chicken pox Cholera	Measles	Mumps	Rubella

Vaccine Derived Poliovirus

- Vaccine associated paralytic poliomyetitis is seen in 4 case/10 lakh birth
- Clinical features are exactly same as that seen with wild poliovirus.
- It is most frequently associated with sabin 3 (60% of cases) followed by sabin 2 and sabin 1.
- In patients with common variable immunodeficiency syndrome, sabin virus replicate for prolong periods, resulting in chronic shedding of vaccine derived poio virus with increased neurovirulence, Such viruses are termed as immunodeficiency associated vaccine derived poliovirus *(iVDPV)*.

Note: HIV or AIDS are risk factors for the development of iVDPV.

2. **Ans. (d) Enterovirus** *Ref. Nelson 18/e, p 2521*

"Enterovirus are the most common cause of viral meningitis and meningoencephalitis in children".

Remember:
- Most common cause of sporadic viral encephalitis: HSV-1
- Most common cause of epidemic viral encephalitis: HSV-1

3. **Ans. (d) Hemorrhagic fever** *Ref. Ananthanarayan 8/e, p 491, 9/e, p 491*

See manifestation of enterovirus in theory of our book.

4. **Ans. (c) Increased muscular activity** *Ref. Ghai 6/e, p 210; Park 22/e, p 185*

Predisposing factors for paralytic polio

- Tonsillectomy
- Tooth extraction
- Strenuous physical exercise
- Cortisone administration
- Intramuscular injection
- Adenoidectomy
- Fatigue

- Oral polio vaccine is recommended to all children below 5 years.
- For eradication it is essential to immunize all infants below 6 months.

5. **Ans. (b) Inactivated vaccine is given IM** *Ref. Park 22/e, p 188*

Inactivated poliovaccine or IPV (salk type) is given subcutaneously or IM.

Other options

Option a

Most cases of polio are asymptomatic

Option c

- **Dose schedule of IPV:**
  - First dose when infant is 6 weeks old
  - Additional doses are recommended prior to school entry and then every five years until the age of 18.

Option d

- These are three serotypes of polio virus
- Most outbreaks of paralytic polio are due to type 1.

6. **Ans. (c) Type I is responsible for vaccine induced paralytic polio myelitis** *Ref. Park 22/e, p 185*

Already explained

7. **Ans. (a) Hemmorrhagic fever** *Ref. Ananthnarayan 8/e, p 491, 9/e, p 491*

See manifestation of enterovirus in theory of our book.

8 (a). **Ans. (b, c, d, e) Stable at pH 4, Cause pleurodynia, Cause encephalitis, Cause meningitis**

Ref. Greenwood 18/e, p 489; Ananthanarayan 9/e, p 491

Let us consider each option

Option a and b

Property	Enterovirus
Capsid form	Icosohedral
– Potypeptide	VP_1, VP_2, VP_3, VP_4
– RNA type	Single stranded positive sense
– RNA molecular weight	2×10^6
Acid	Stable (pH 3-9)
Optimal temperature	37°C
Density in caesium chloride	1.34

Option c, d, e

Diseases associated with enterovirus	
Paralysis	Poliovirus, Coxsackie A
Aseptic meningitis	Poliovirus Coxsackie A, Coxsackie B, ECHO
Encephalitis	Poliovirus Coxsackie A, Coxsackie B, ECHO
Fever with rash	Coxsackie A, ECHO

Diseases associated with enterovirus	
Hand foot and mouth disease	Coxsackie A
Herpangina	Coxsackie A
URI	Coxsackie A
Pneumonia, bronchiolitis	New enterovirus types
Myocarditis, pericarditis	Coxsackie B virus
Acute hemorrhagic conjunctivitis	Cox Sackie A
Bornholm disease	Cox Sackie B virus

8 (b). **Ans. (a) Staphylococcus** *Ref. Harrison 19/e, p 1212*

Pulmonary complications of influenzae

a. **Primary influenza viral pneumonia:** Least common but most severe of all pulmonary complications of influenzae Sputum production is generally scanty, but sputum may contain blood. In advanced cases, diffuse rates may be noted X-ray finding shows diffuse interstitial inflitrates. Primary influenzae pneumonia has predilection for individuals with cardiac diseases.

b. **Secondary bacterial pneumonia:** Characterised by reapperance of fever along with sign and symptoms of bacaterial pneumonia after initial improvement. The commonest bacterial pathogens are S.pneumoniae, staphylococcus aureus, and haemophilus influenzae.

c. **Mixed bacterial and viral pneumonia:** Most common pneumoinc complication after influenza patient may show gradual progression of their acute illness or may show transient improvement followed by clinical exacerbation. Patients with mixed viral and bacterial pneumonia have less wide spread involvemnet of lung than those with primary viral pneumonia

d. Worsening and exacerbation of COPD

e. **Other pumonary complications:** Worsening of COPD, In children croup, sinsusitis as well as otitis media can be there

9. **Ans. (a) Bird flu virus** *Ref. Park 22/e, p 147*

H_5N_1 is a type of new influenza virus which is a causative agent of bird flu.

- Majority of avian influenza do not infect humans. However, avian H5N1 is a strain with pandemic potential since it ultimately adapt into a strain that is contagious among humans.

10. **Ans. (d) Hemagglutinin and neuraminidase are strain specific** *Ref. Ananthanarayan 8/e, 495' 9/e p 497-498*

Antigenic Structure of Influenza Virus	
Surface/viral or V antigen	**Internal antigen**
• Strain specific	a. Envelop antigen (nucleocapsid) - Host specific
• Shows antigenic variation	b. Membrane (M) antigen - Type specific, i.e. A, B or C
• **Two types:** a Hemagglutination - Antibody against this is protective b Neuraminidase - Antibody against this is not protective	c. Ribonucleoprotein (RNP) or soluble(s) antigen: – Type specific – Stable, i.e. not shows antigenic variation

Antigenic variation	
Antigenic drift or Minor antigenic variation	***Antigenic shift or Major antigenic variation***
Due to point mutation	Due to genetic reassortment
Responsible for epidemic	Responsible for pandemics
Shown by A, B	Only shown by type A

Remember:
- Ds RNA – Reoviridae family (reo, orbi, rota virus), Picobirnaviruses
- Ss DNA – Parvoviridae

11. Ans. (a) Influenza virus *Ref. Harrison 17/e, p 1080, 18/e, p 1493*

Segmented RNA viruses are:	
P – Picobirna viruses	→ ds RNA
A – Arena viridae	→ ds RNA
R – Reoviridae	→ ds RNA
B – Bunyaviridae	→ ss RNA
O – Orthomyxoviridae (Influenza)	→ ss RNA

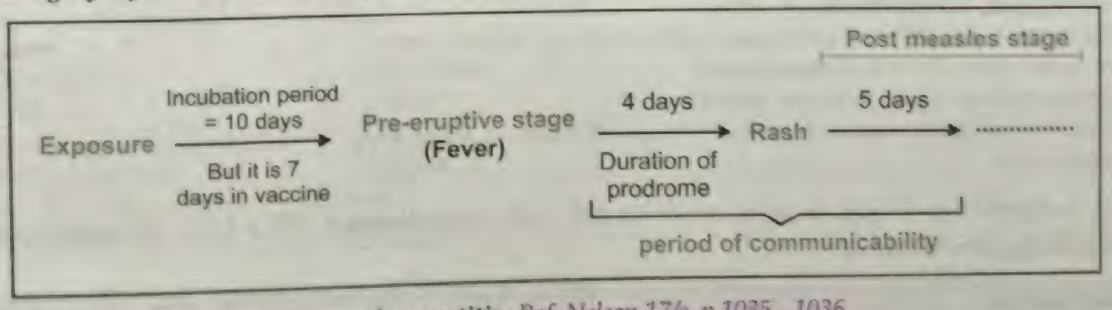
Mnemonic = PARBO

12. Ans. (a), (b) and (c) Influenza, Cholera and Plague *See below:*

Disease	Incubation Period
Pertussis	7 - 14 days
Chicken pox	14 - 16 days
Polio	7 - 14 days
Rubella	2 - 3 weeks (average 18 days)
Mumps	usually 18 days
Influenza	18 - 72 hrs
Diptheria	2 - 6 days
Menigococcal meningitis	usually 3 - 4 days
Cholera	a few hours up to 5 days (commonly 1 - 2 days)
Rabies	highly variable, commonly 3 - 8 weeks (vary from 4 days to many years)
Plague	
- Bubonic plague	2 - 7 days
- Septicemic plague	2 - 7 days
- Pneumonic plague	1 - 3 days
Tetanus	usually 6 - 10 days
Leprosy	average 3 - 5 years or more

13. Ans. (c) Not infectious in prodromal period *Ref. Park 22/e p 138*

"Measles is highly infectious during the prodromal period and the stage of rash."



14. Ans. (a) Meningoencephalitis can precede parotitis *Ref. Nelson 17/e, p 1035 - 1036*

Important Points About Mumps

- *MC* manifestation of mumps - bilateral parotitis.
- It can also involve submaxillary and sublingual glands but never involved alone.
- *MC* manifestation (other than parotitis) in post pubertal males is orchitis (sterility is rare). Seen in 20% of cases.
- Though sterility is rare, subfertility is estimated to occur in 13% of cases of unilateral orchitis and in 30-87 of cases of bilateral orchitis.
- Oophoritis is far less common than orchitis. It also not lead to sterility.
- *MC* manifestation (other than parotitis) in children: Aseptic meningitis which may develop before, during or in absence of parotitis.
- **Period of communicability** – Usually 4-6 days *before onset of symptoms and a week or more* thereafter. Period of maximum infectivity is just before and at onset of parotitis.

Complications of Mumps

- ***Meningoencephalomyelitis***
  - *MC* complication in childhood.
  - Males are affected *more commonly.*

- May be either due to primary infection of neuron or post-infectious encephalitis with demyelination.
- In primary infection it occurs before parotitis while in post-infectious form, it follows parotitis.
- Parotitis may be absent in some cases.
- CSF shows lymphocytic pleocytosis.

- ***Orchitis and Epididymitis***
  - These complications are rare in prepubscent age group, while common inadolescent and adults.
  - Infertility is rare even with bilateral orchitis.
- Pancreatitis, myocarditis, arthritis, thyroiditis, Mastitis, GB syndrome, Transverse myelitis
- Cerebral ataxia, measles associated deafness, dacryoadenitis are other complications.

15. Ans. (a) Orchitis and Oophoritis *Ref. Harrison 17/e, p 1220, 18/e, p 1609; Park 22/e p 143*
Already explained

16. Ans. (b) Mumps cause *Ref. Harrison 17/e, p 1220, 18/e, p 1609*
Already explained

17. Ans. (d) SSPE *Ref. Harrison 17/e, p 1215, 18/e, p 1603*

Complication of Measles	
• *Otitis media (MC)* • *Pneumonia*	Very common in infants with measles May be primary viral pneumonia or bacterial superinfection; frequent reason for hospitalization of adults; measles rash sometimes lacking in immunocompromised patients with measles pneumonia. Primary giant cell *(Hecht's)* pneumonia is seen in immunocompromised
• *Croup*	Occasionally severe
• *Gastroenteritis*	Many children with measles develop diarrhea which contributes to malnutrition
• *Cervical adenitis*	Due to lymphoid hyperplasia as host response to virus; common
• *Acute encephalitis*	May be mild to severe/fatal; occurs in 1 in 1000 cases of mesles
• *Subacute sclerosing panencephalitis (SSPE)*	In 1 in 100,000 cases of measles, usually when measles occured in infancy; seen 5-10 years later. SSPE is a rare progressive disease characterized by seizures and progressive deterioration of cognitive and motor functions and death

18a. Ans. (d) i.e. Norovirus *Ref. Harrison 19/e, p 1285*
"Norovirus may be the most common infectious agent of mild gastroentreritis in the community and affects all age groups."
Norovirus

- Non enveloped, Icosahedral RNA virus
- Infectious dose: 10–100 viral particles
- Route of infection: Fecoral, aerosolization
- Incubation period: 12–72 h, illness lasts 12–60 h
- *Clinical features*: Loose waters stools without blood, mucous, leucocytes.
- *Diagnosis*: PCR

18b. Ans. (a) i.e. Antigen in stool *Ref. Ananthanarayan 8/e, p 557, 9/e, p 560; Harrison 19/e, p 1289, 18/e, p 1592*
Diagnosis of Rotaviurs Diarrhea

1. Stool examination
• Genotyping of rotavirus nucleic acid by PCR is most sensitive method.
• As virus is shed in large quantities (10^7-10^{12}g) in stool, diagnosis is confirmed by detecting *virus in faeces* by enzyme immunoassay or viral RNA can be detected by gel electrophoresis, probe hybridization, or PCR.
• Viral shedding detectable by EIA usually subsides within a week but may persist for >30 days in immunocompromised while PCR detect viral shedding for longer periods.
• Electron microscopy / immunoelectronmicroscopy is used to see virus in feces. Conc. > 10^8 virus/ml is necessary
• Serological techniques for demonstration of stool are simple and sensitive. Rotavirus share a common group antigen situated in the inner capsid layer which can be detected by IEM, ELISA or latex agglutination.
• They do not grow readily on cell culture.
2. Serology: IgM or IgG antibodies in the blood are increased.

19. Ans. (a) Rotavirus *Ref. Harrison 19/e, p 1289, 18/e, p 1592*

- The first rotavirus vaccine was introduced in 1998 and withdrawn because it was linked with intussuception.
- In 2006 two new rotavirus vaccines have been introduced, one of this is a multivalent bovine human reassortant rotavirus preparation. Second one is a single attenuated rotavirus strain.

20. **Ans. (a), (c) Herpes, Rotavirus** *Ref. Harrison 17/e, p 1206, 18/e, p 1591*

Reassortment

Mixing of the genetic material of different strains of a species in to a new combination. Process contributing of re-assortment includes:

- Assortment of chromosomes
- Chromosomal crossover

It is typically seen in influenza virus and rota virus. E.g. If a single host (human or chicken) in infected by two different strains of the influenza virus, then it is possible that new assembled viral particle may have segment from both the strains.

21. **Ans. (b) Rabies** *Ref. Ananthanarayan 8/e 432, 9/e, p 452*

- **Vaccine that grows in embryonated eggs:**
  - Influenza
  - Yellow fever (17 D strain)
  - Rabies (Flury strain)
  - ***Mumps***
- Varicella vaccine growns in chick embryo fibroblast culture.
- Rubella – RA 27/3 vaccine produced in human diploid fibroblast. ... *Park 22/e, p 142*
- No eggs culture vaccine of measles are produced. All are tissue culture vaccine, either chick embryo or human diploid cell line.

22. **Ans. (b) Rabies** *Ref. Harrison 19/e, p 1301, 18/e, p 1612*

- Negri bodies are the intracytoplasmic inclusions of rabies virus in the CNS.
- Negri bodies are distributed throughout the brain particularly in Ammon's horn, the cerebral cortex, the brain stem, the hypothalamus, purkinje cells of cerebellum and the dorsal root spinal ganglia.
- They are not seen in about 20% cases of rabies and their absence does not rule out the diagnosis.

Inclusion Bodies		
It is of following types:		
a. Intracytoplasmic eosinophilic inclusions:		
Negri bodies	–	rabies
Guarnieri bodies	–	variola (small pox), vaccinia
Bollinger bodies	–	fowlpox
Henderson – Peterson bodies	–	molluscum contagiosum
b. Intranuclear acidophilic inclusion bodies:		
Cowdry type A	–	herpes, chicken pox, CMV, yellow fever
Torres bodies	–	yellow fever
Cowdry type B	–	polio virus
c. Both Nuclear and cytoplasmic:		
Warthin Finkeldey	–	measles
d. Intranuclear basophilic inclusion bodies:		
Cowdry type B	–	adenovirus

23. **Ans. (c) Immediately stitch wound under antibiotic coverage** *Ref. Park 22/e, p 254; 21/e, p 253-254*

"Immediate stitching is contraindicated."

- **Combined administration of:** Single dose of anti-rabies serum; Anti-rabies vaccine (ARV) and; Local treatment of wound is the *best* specific prophylactic treatment after exposure of man to rabies (especially in all severe cases, i.e. class III exposures and in all cases of unprovoked bites by wild animals).
- **Anti-Rabies Serum *(Passive immunity)*** should be given as promptly as possible after sensitivity test, irrespective of interval between exposure and begining of treatment.
  - It prolongs I.P. if administered soon after exposure to rabies so it is particularly important in class III bites.
  - It is given either as Horse anti-rabies serum (40 IU) or Human rabies immunoglobulin (20 IU) in which part of the dose is given around the wound and rest by IM in gluteal region.
- **Local Treatment of wound:**
  - It can reduce the chances of developing rabies by up to 80%. It consist of:
    a. Cleansing with plenty of soap and water, preferably under a running tap for at least 5 minutes.
    b. Chemical treatment by virucidal agents either alcohol, tincture 0.01% of aqueous solution of iodine or povidone iodine (not use savlon, cetavlon, carbolic or nitric acid).
    c. ***Suturing - Not done immediately*** but should be done 24 - 48 hours later.

24. Ans. (b) Licks on Fresh wound *Ref. Park 22/e, p 254*

Classification of Exposures		
Class I (Slight risk)	***Class II (Moderate risk)***	***Class III (Severe risk)***
• Licks on healthy unbroken skin • Consumption of unboiled milk of suspected animal • Scratches without oozing of blood	• Licks on fresh cuts • Scratches with oozing of blood • All bites except on head, neck, face, palm, fingers • Minor wounds less than 5 in number	• All bites or scratches with oozing of blood on neck, head, face, palm, fingers • Lacerated wounds on any part of body • Multiple wounds 5 or more in number • Bites from wild animals

25. Ans. (c) RNA has a negative polarity *Ref. Ananthanarayan 7/e, p 535-36, 9/e, p 530-532*

Important features of Rabies virus

- **Bullet** shaped
- Belong to rhabdoviridae-serotype 1 (Lyssavirus type - 1) [Serotypes 2, 3 and 4 are rabies related viruses].
- Lipoprotein envelope carry glycoprotein spikes.
- Core consists of helically arranged ribonucleoprotein and **RNA dependent RNA transcriptase.**
- Genome is unsegmented, linear, negative sense RNA **[Other-ve sense RNA viruses - Ortho, paramyxoviridae].**
- Virus is inactivated by phenol, formalin, betapropioplactone, ultraviolet, irradiation, sunlight.
- Virus has hemagglutinating activity due to spikes.
- Virus spreads centripetally from site of inoculation *(within striated muscle)*, than ascends throgh nerve associated tissue space and than spreads centrifugally in peripheral autonomic nerves to many tissues including salivary glands. Thus pathogenesis does not show that it has affinity for motor neurons.

26. Ans. (a) Incubation period depends on the site of bite *Ref. Park 22/e, p 252; Ananthanarayan 8/e, 528, 9/e, p 532*

Incubation period of Rabies depends on the:

- Site of bite
- Number of wounds
- Species of biting animal
- Severity of bite
- Amount of virus injected
- Protection provided by clothing and treatment undertaken

- *I.P is shorter in:*
  - Severe exposures
  - Bites on face, head, neck and upper extremities
  - Bites by wild animals.
- In India most of human rabies cases have resulted from dog bites but it ***also occurs by bite of cat, monkey, horse, Jackals, fox-hyena, etc.***
- Also transmitted by licks on abraded skin and mucosa; aerosols; corneal and organ transplants.
- Characteristic of rabies is Negri Body which is ovoid eosinophilic intracytoplasmic inclusion bodies.
- Rabies is bullet shaped SS negative sense RNA virus.

27. Ans. (b) Corneal impression smear for immunofluoresence stain *Ref. Ananthanarayan 8/e, 529, 9/e, p 535*

"The method most commonly used for diagnosis of rabies is demonstration of rabies virus antigen by immunofluorescence. The specimens tested are corneal smears and skin biopsy (from face or neck) or saliva in live cases, and brain postmortem".

Diagnosis of Human Rabies

- *Specimen*
  (a) *Antemortem* Corneal smears, skin biopsy from face or neck, saliva.
  (b) *Postmortem:* Brain
- Method
  - *Most commonly used* for diagnosis is the demonstration of rabies virus ***antigens by immunofluorescence*** (direct or using monoclonal antibodies).
  - Demonstration of negri bodies in the brain or spinal cord.
  - ***Isolation*** of virus by intracerebral inoculation in mice; from the brain, CSF, saliva, urine.
  - Rapid isolation is done by tissue culture cell lines.
  - High titre rabies specific ***antibodies in CSF*** (Not seen after immunization) by Fluorescent antibody test.
  - Detection of rabies virus RNA in saliva by Reverse transcription PCR.

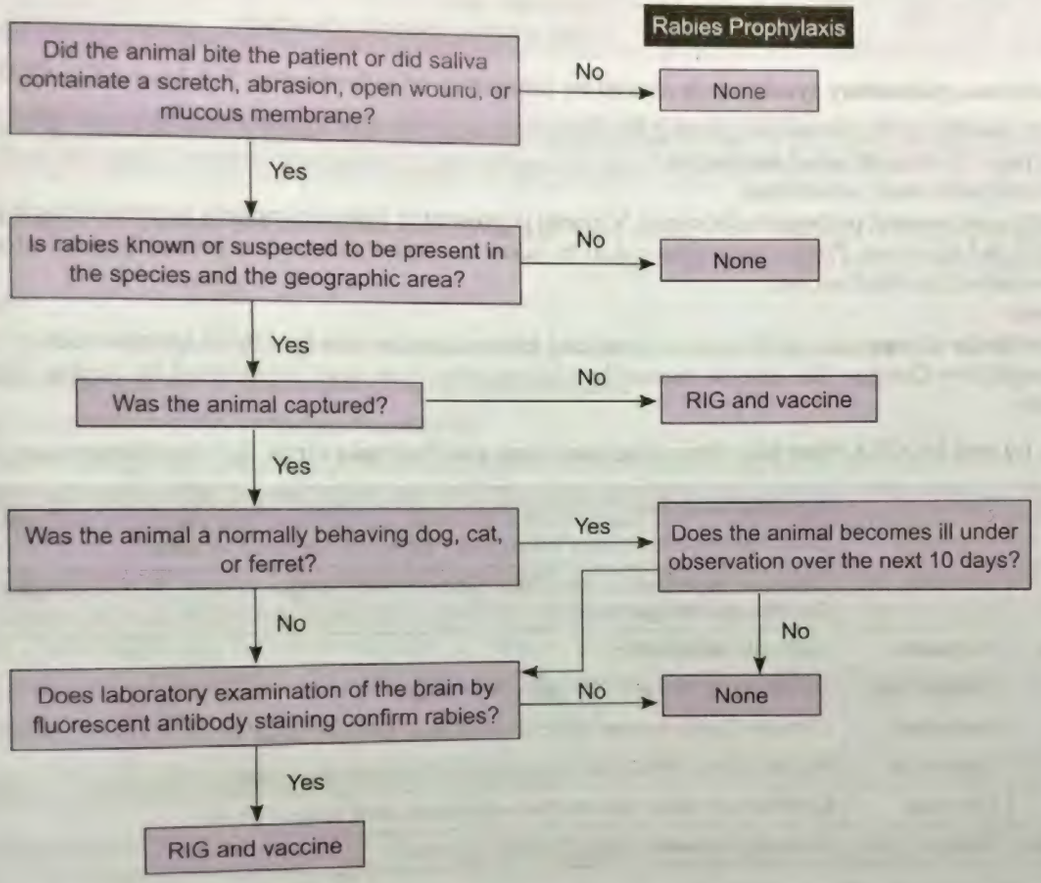
Remember:
- Rabies infection terminates in death, not lifelong immunity.
- Rabies virus has single serotype, i.e. Lyssavirus type 1.
- Rabies vaccine is killed inactivated vaccine.
  - Inactivation is commonly done by phenol or Betapropiolactone.

28(i). Ans. (b) With hold... *Ref. Harrison 18/e, 1615-1616, 19/e, p 1302-1304*

"Healthy dogs, cats, or ferrets may be confined and observed for 10 days. Post exposure prophylaxis is not necessary if animal remains healthy. If the animal develop signs of rabies during the observation period, it should be euthanized immediately, and the head should be transported to the laboratory under refrigration and examined for the presence of rabies virus by DFA testing."

...Harrison 19/e 1303

In high risk exposures and in areas where canine rabies is endemic rabies prophylaxis should be initiated without waiting for lab results.



28(ii). Ans. (b) Negri bodies *Ref. Harrison 19/e, p 1301, 18/e, p 1612*

Already explained

28(iii). Ans. (c) Antibodies to rabies virus

Corneal Smear test

- Rabies virus antigen can be detected by simple FAT test on corneal epithelium during the terminal stages.
- The methodology of this test involves the gentle rubbing of that surface of a clean microscope glass slide on each cornea.
- The test is not routinely performed due to its low sensitivity.

29. Ans. (a) Relapsing fever *Ref. Park 22/e, p 712, 725; 21/e, p 720-721*

Tick borne diseases		
Hard tick	• Tick typhus • Tularemia • Human Babesiosis • Viral encephalitis *(not Japenese encephalitis which is transmitted by culex)*	• Viral encephalitis • Tick paralysis • Colorado tick fever • Viral hemorrhagic fever *(e.g. Kyasanur forest disease)* • Q fever
Soft tick	• Relapsing fever	

30. Ans. (c) Kyasanur Forest disease *Ref. Park 22/e, p 725; 21/e, p 720-721*

Already explained

31. Ans. (c) KFD is transmitted by ticks *Ref. Park 22/e, p 262-263*

- *Yellow fever* is an exotic disease for India, i.e. disease which may be imported in India or India is yellow fever 'receptive' area that is "an area in which yellow fever does not exist but where condition would permit its development if introduced".
- *Dengue virus* has at least **4 *serotypes*** (not one).
- ***Japanese encephalitis*** is transmitted by ***culex*** (not Aedes).

Remember: Other culex transmitted diseases are:
- Bancroftian filariasis
- West Nile fever
- Viral arthritis (epidemic/polyarthritis).

32. Ans. (a) Hantavirus pulmonary syndrome is caused by inhalation of rodent urine *Ref. Jawetz 25/e, p 531; Park 22/e, p 262*

Hantavirus are classified in the hantavirus genus of the Bunyaviridae family. It can cause very serious and often fatal disease.

(a) Hemorrhagic fever with renal syndrome.
(b) Hantavirus pulmonary syndrome.

- Hantavirus are natural pathogen of rodents. Viremia is present in infected rodents and the virus is shed in urine, feces and saliva in high titres. *Transmission from rodent to rodent and rodent to human* is primarily respiratory by inhalation of virus contained in dried excreta.

Other options:
- *KFD* is a febrile disease caused by an arbovirus and transmitted to man by bite of infective tick.
- *Chikugunya fever*-Dengue like disease caused by chikugunya virus and transmitted by Aedes, Culex and Mansonia mosquito.

33. Ans. (a), (b), (c) and (e) KFD; West Nile fever; Ganjam virus; and Puumala virus *Ref. Ananthanarayan 8/e, p 513, 9/e, p 517*

Family	Genus	Important species
Togaviridae	Alphavirus	Chikungunya, Sindbis and Venezuelan equine encephalitis viruses
Flaviviridae	Flavivirus	Japaness encephalitis, West Nile, Yellow Fever, Dengue types 1, 2, 3, 4, Kyasanur Forest Disease, Omask hemorrhagic fever
Bunyaviridae	Bunyavirus	California encephalitis
	Phlebovirus	Sandfly fever viruses, Rift valley fever virus
	Nairovirus	Crimean Congo hemorrhagic fever viruses, Ganjam virus
	Hantavirus	Hantan, Seoul, Puumala, Prospect Hill, Sin Nombre viruses
Reoviridae	Orbivirus	Colorado tick fever, African horse sickness, Blue tongue viruses
Rhabdoviridae	Vesiculovirus	Vesicular stomatitis virus, Chandipura virus

34. Ans. (a) Amplifier *Ref. Park 22/e, p 259-260*

Japanese encephalitis
- Caused by Group B arborius (filovirus)
- **Vector**: – *Culex triteniorhynchus* (most important)
  – *C. vishnvi*
  – *C. gelidus.*
- Man is an incidental ***'dead end'*** host.
- **Animal host**: – Pigs – major vertebrate host. – not manifest any symptoms so act as amplifiers.
  – Cattles and buffaloes: Act as mosquito attractants.
  – *Horses* – only domestic animals which show signs of encephalitis.
- **Birds host** – Pond herons (reservoir host), Cattle egrets, poultry and ducks.
- ***Incubation period in man*** – 5-15 days.
- Average period between onset of illnes and death is about 9 days.
- ***Diagnosis*** is mainly based on serology using ***IgM capture ELISA***.

Remember: Killed mouse brain vaccine is available by which immunity develops after one month of second dose.

35. Ans (a) IgM ELISA *Ref. Harrison 18/e, p 1621; Park 22/e, p 228-229*

Laboratory diagnosis of Dengue
- Thrombocytopenia (100,000/mm^3 or less)
- *Hemoconcentration:* Hematocrit increased by 20% or more of base line value

- Leukopenia
- Elevation of serum Aminotransferase (±)
- Antigen detection ELISA or RT PCR or virus isolation in acute phase
- IgM ELISA or paired serology during recovery
- Strip immunochromatographic test of IgM for rapid diagnosis

Remember: IgM appears within 2-5 days of onset of illness and persists for one to three months.

36. Ans (d) Hand-foot-mouth disease *Ref. Ananthanarayan 7/e, p 522, 9/e, p 491*
Already Explained

Remember: Hand, Foot and Mouth Disease (HFMD) is caused by coxsackie A-16, 9; B 1-3; Enterovirus 71: It is an exanthematous fever of young children characterized by papulovesicular lesion on skin and oral mucosa.

37a. Ans (a, c, d) Caused by 4 serotypes, Presents with fever and joint pain, Virus belongs to flavivirus genus
Ref. Ananthanarayan 9/e, p 525; Greenwood 18/e, p 531-532

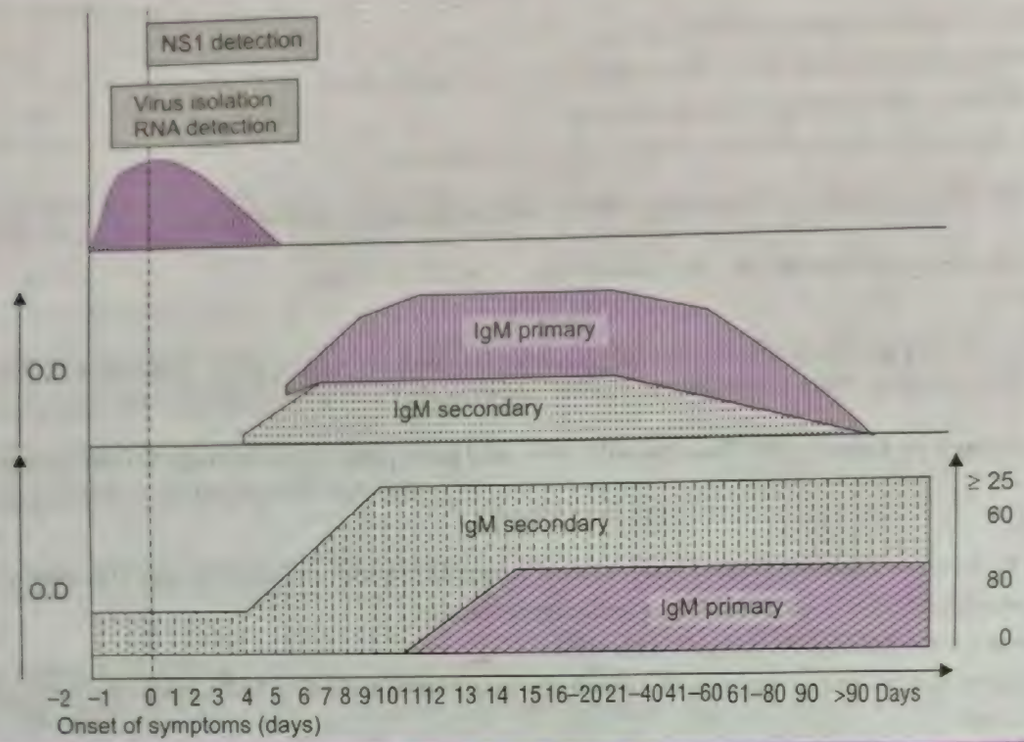
Dengu Fever

- Caused by four serologically related virus termed dengu 1, 2, 3 4. Out of 4 types, type II is most virulent
- All are enveloped SS RNA virus belongs to flavivirus family
- Diseases is transmitted by ***Ae. aegypti mosquito***
- Epidemics of dengue are common in Southeast Asian countries including India, particularly in September-October.
- *IP* : 3-14 days
- Dengue present clinically as fever of sudden onset with headache, retrobullar pain, pain in back and limbs ***(break bone fever)***, lymphadenopathy and maculopapular rash. The fever is usually biphasic that lasts for 5-7 days
- In some cases (which exhibit hypersensitive or enhance response) dengue may present with hemorrhagic manifestation (dengue hemorrhagic fever) or with shock (dengue shock syndrome)
- Control of dengue is limited to vector control as **no vaccine** is currently available
- ***No vertebral hosts other than humans*** have been identified.

37b. Ans (d) ELISA for antibody detection against dengue virus

Laboratory diagnosis of Dengue

- ***Detection of Virus:*** After the onset of illness, the virus can be detected in serum, plasma, circulating blood cells and other tissues for 4–5 days. During the early stages of the disease, virus isolation, nucleic acid or antigen detection can be used to diagnose the infection.
- ***Nucleic Acid Amplification Test:*** Before day 5 of illness, during the febrile period, dengue infections may be diagnosed by detection of viral RNA by nucleic acid amplification tests (NAAT). Nucleic acid detection assays with excellent performance characteristics may identify dengue viral RNA within 24–48 hours
- ***Antigen Detection Test:*** Detection of viral antigens by ELISA can be used in first week of disease. NS1 antigen detection kits have now become the commonest method for diagnosis of dengue. After day 5, dengue viruses and antigens disappear from the blood coincident.
- ***Serology:*** Method of choice at the end of infection. Antibody response to infection differs according to the immune status of the host
  a. *Primary dengue infection* (in persons who have not previously been infected with a flavivirus or immunized with a flavivirus vaccine): IgM antibodies are the first immunoglobulin isotype to appear. They are detectable in 50% of patients by days 3-5 after onset of illness, increasing to 80% by day 5 and 99% by day 10. IgM levels peak about two weeks after the onset of symptoms and then decline generally to undetectable levels over 2–3 months.
  b. *Secondary dengue infection* (a dengue infection in a host that has previously been infected by a dengue virus, or sometimes after non-dengue flavivirus vaccination or infection): Antibody titres rise rapidly the dominant immunoglobulin isotype is IgG and is detectable at high levels.
- To distinguish primary and secondary dengue infections, IgM/IgG antibody ratios are now more commonly used than the haemagglutination-inhibition test (HI)



Diagnostic methods	Diagnosis of acute infection	Time to results	Specimen	Time of collection (days after onset of symptoms)
Viral isolation and serotype identification	Confirmed	1-2 weeks	Whole blood, serum, tissues	1-5 days
Nucleic acid detection	Confirmed	1 or 2 days	Tissues, whole blod, serum, plasma	1-5 days
Antigen detection	Not yet determined Confirmed	1 day > 1 day	Serum Tissue for immuno-chemistry	1-6 days NA
IgM ELISA	Probable	1-2 days	Serum, plasma, whole blood	After 5 days
IgM rapid test		30 minutes		
IgG (paired sera) by ELISA, HI or neutralization test	Confirmed	7 days or more	Serum, plasma, whole blood	Acute sena, 1-5 days: convalescent after 15 days

38. **Ans. (d) Respiratory syncytial virus** *Ref. OP Ghai 6/e, p 352; Nelson Pediatrics 17/e, p 1076*

- RSV is the *most common* cause of bronchiolitis.
- RSV is the *most common* cause of viral pneumonia in infants.

RSV

- RNA virus belonging to family paramyxoviridae.
- In infants *MC* maifestation is ***bronchiolitis.***
- In adults *MC* manifestation is ***common cold.***

Remember: Treatment of Bronchiolitis:

- In uncomplicated cases, treatment is symptomatic.
- Humidified oxygen is usually indicated for hospitalized infant.
- Epinephrine may be given, however, corticosteroids are not indicated.
- Antiviral drug ribavirin has moderate efficacy.

39. **Ans. (d) Infection after 16 weeks of gestation results in major congenital defects**

Ref. Park 21/e, p 140-41; 22/e, p 141-142; Harrison 19/e, p 230, e-1; 18/e, p 1605-1606

Important features of Rubella virus

- Rubella is RNA virus of ***togavirus*** family.
- No known carrier state for postnatally acquired rubella.
- Infectivity is greatest when rash is erupted.

- Rubella (*German measles*) is mainly a disease of childhood, particularly 3-10 years.
- One attack results *life-long immunity*
- It causes two types of disease:

a. Postnatally Acquired Rubella

- Virus is shed from pharynx during prodromal phase and continue for about a week after onset.
- It is invariably self-limited.
- *Symptoms*
  - Posterior auricular, cervical and suboccipital lymphadenopathy; fever and rash *(begins on face and spreads down the body)*.
  - Petechial enanthem on soft plate called *Forschheimer* spots may be seen.
- **Complications:**
  - Arthiritis (*MC* in fingers, wrist or knees) almost exclusively in women.
  - Hemorrhage due to thrombocytopenia and vascular damage.
  - Encephalitis (*in immunosuppressed*)
- **Diagnosis:**
  - Throat swab culture for virus isolation
  - **Serology**: - Most widely used serological test is hemagglutination inhibition test (HAI).
    - 4-fold rise in HI antibody titer in paired sera or presence of IgM in single sera obtained 2-weeks after the rash is **diagnostic** of recent rubella infection.

b. Congenital Rubella Syndrome

- Infectivity as well as severity is more in early pregnancy *(first trimester of <11 weeks)*.
- *Classic triad of patent ductus arteriosus (cardiac malformation), cataract* and *deafness* is seen.
- Infection in 2nd trimester - may be deafness only.
- >16 wks – no major abnormalities
- **Diagnosis**
  - Isolation of virus in cell cultures of throat samples, urine or other secretions.
  - Detection of IgM in single serum sample shortly after birth.
  - Persistance of Rubella IgG antibodies serum beyond 1 year or rising antibody titer anytime during infancy in an unvaccinated child.
  - Biopsy of tissues/blood/CSF fluid for viral antigen by monoclonal antibodies.
  - Detection of Rubella RNA by in situ hybridization and PCR.
- **Prevention:**
  - **Rubella vaccine:** Live attenuated RA 27/3 vaccine
  - **Strategy:**
    - Immunize all infants at 12-15 mths with MMR and second dose in early childhood
    - Also administer to anyone who is thought to be susceptible to infection and is not pregnant.
    - **Contraindication for Rubella vaccine:** Pregnancy

Remember: Pregnancy should be avoided for at least 3 months after rubella vaccination.

40. Ans. (d) Detection of viral hemagglutination inhibiting (HAI) antibodies in a single serum specimen

Ref. Ananthanarayan 9/e, p 449-450, 457; 8/e, p 448-49

Laboratory Diagnosis of Viral Disease

- ***Microscopy:*** By electron microscopy (for viral diarrhea); Fluorescent antibody technique (e.g. Rabies), etc.
- ***Demonstration of virus antigen:*** By counterimmunoelectrophoresis, radioimmunoassay, ELISA, precipitation in gel immunofluorescence.
- ***Isolation of virus:*** – By inoculation into animals, eggs or tissue culture.
  – As most viruses are heat labile, refrigeration is essential during transport.
- ***Serological diagnosis:***
  - By neutralization, complement fixation, ELISA, Hemagglutination inhibition tests, immunofluorescence.
  - It is essential to examine paired sera (acute and convalescent).
  - Examination of single sample of serum for antibodies is meaningful only when IgM, specific test are done.
  - Serological diagnosis is based on greater than fourfold rise in IgG in convalescent sera when acute and convalescent serum are analyzed at the same time. A simultaneous fall in IgM confirms recent primary viral infection.
  - Paired sera with rising titers of antibody to virus specific antigens and shift from IgM to IgG are generally accepted as diagnostic of acute viral infection.
  - Hemadsorption and hemagglutination assay measure the ability of serum antibodies to inhibit RNA virus induced erythocyte adsorption or agglutination.

41. **Ans. (a) 6-12 weeks of pregnancy** *Ref. Harrison 17/e, p 1219, 18/e, p 1606*
Already explained

42. **Ans. (c) Respiratory syncytial virus** *Ref. Ghai 6/e, p 352-353*
"Child of 11 month (infant) with respiratory distress, bilateral crepitation and wheezing is a typical presentation of Bronchiolitis."

Cause of Bronchiolitis	
Respiratory syncytial virus (MC)	
Parainfluenza 3, 1, 2	
Adenovirus	
Influenza virus	
Mycoplasma pneumonia (Rarely).	Mnemonic - **My PAIR**

43. **Ans. (b) HEV** *Ref. Ananthanarayan 8/e p 439, 9/e, p 439*

Picornaviruses		
Enterovirus	**Rhinoviruses**	**Hepatoviruses**
↓ Polio, Coxasackie, Echo, Other enteroviruses		↓ • HAV (enterovirus 72)

Foot and mouth disease is manifestation of enterovirus and coxsackie.

44. **Ans. (c) and (d) Measles - Edmonston zugreb strain and BCG-Danish 33 strain** *See below:*

Vaccine	Strain
Mumps	Jeryl-Lynn strain
Chicken pox	OKA strain
Measles	HDC-Edmonston-Zagreb strain
Rubella	RA 27/3
BCG	Danish 1331 Strain
Cholera	CVD. 103 - HgR strain
Typhoral	Ty 21-a strain
Yellow fever 17-D	Asibi strain

Non-cultivable viruses:
- Rotavirus (certain strains can grow in tissue culture)
- Norwalk virus
- Mollascum contagiosum
- Hepatitis virus except hepatitis A

45. **Ans. (a) and (c) Smallpox virus and Ebola virus** *Ref. Harrison 18/e, p 1769*

Bioterrorism agents
- **Category A:**
  - Anthrax *(Bacillus anthracis)*
  - Botulism *(Clostridium botulinum toxin)*
  - Plague *(Yersinia pestis)*
  - Smallpox *(Variola major)*
  - Tularemia *(Francisella tularensis)*
  - Viral hemorrhagic fevers:

Arenaviruses:	Lassa, New World (Machupo, Junin, Guanarito, and Sabia).
Bunyaviridae:	Crimean Congo, Rift Valley
Filoviridiae:	Ebola, Marburg
Flaviviridae:	Yellow fever, Omsk fever, Kyasanur Forest

- **Category B:**
  - Brucellosis *(Brucella spp.)*
  - Epsilon toxin of *Clostridium perfringens*
  - Food safety threats *(e.g., Salmonella spp., Escherichia coli 0157: H7, Shigella)*
  - Glanders *(Burkholderia mallei)*
  - Melioidosis *(B. pseuodmallei)*
  - Psittacosis *(Chalmydia psittaci)*
  - Q fever *(Coxiella burnetii)*
  - Ricin toxin from *Ricinus communis* (castor beans)
  - Staphylococcal enterotoxin B
  - Typhus fever *(Rickettsia prowazekii)*
  - Viral encephalitis [alphaviruses (e.g. Venezuelan, eastern, and western equine encephalitis)]
  - Water safety threats *(e.g. Vibrio cholerae, Cryptosporidium parvum).*
- **Category C:**
  - Emerging infectious diseases threats such as nipah, hantavirus and SARS coronavirus.

46. **Ans. (a), (c) and (d) RSV, Parainfluenza and Influenza** *Ref. Ghai 6/e, p 352-353*
Already explained

47. **Ans. (c) Simian 40** *Ref. Ananthanarayan 9/e, p 552, 562, 8/e, p 549*

SV 40 = Simian vacuolating virus 40 = Simian virus 40

- It is a DNA viruses
- Its family is papovaviridae and genera is polyoma virus.
- It is oncogenic virus, causing cytopathic effects with prominent cytoplasmic vacoulation

Remember: Simian virises 5, 41 are animal virus antigenically similar to paranfluenza virus type 2

48. **Ans. (a) DNA virus** *Ref. Ananthanarayan 8/e, p 522, 9/e, p 527*

- Hantaan virus is an RNA virus belong to genus Hantavirus and family Bunya viridiae.
- It causes two syndromes:
  - a. ***Hemorrhagic fever with renal syndrome (HFRS) or Manchurian epidemic HF or rodent bone nephropathy***
    - It occurs in two forms:
      - i. Epidemic nephritis–milder form
      - ii. Epidemic hemorrhagic fever–serious form
    - Resembles typhoid, leptospirosis and scrub typhus clinically.
    - Genus hantavirus contain four species–Hantaan, Seoul, puumala and Hill virus.
    - They are natural pathogen of rodents so considered as robovirus and not stirctly an arbovirus infection.
    - ***Major host*** for hantaan: Field mice
      - Transmission from rodent to rodent and rodent to human is primarily respiratory, by inhalation of virus con tained in dried excreta.
    - ***Diagnosis:*** Demonstrating IgM by ELISA or of rising titre of immune adherence hemagglutinating antibodies in paired sera.
  - b. ***Hantavirus pulmonary syndrome***
    - Caused by new Hantavirus the *Sin Nombre* (meaning nameless) virus which is associated with deer, mouse and other rodents.
    - ***No arbovirus*** is linked in transmission.
    - Transmission occurs by inhalation of virus areosol in dried rodent feces.

49. **Ans. (a), (c) and (d) Nipah virus, Corona virus and SARS** *Ref. Harrison 18/e, p 1769*
Already explained, **(category C)**

50. **Ans. (a), (c) and (d) Hantaviurs pulmonary syndrome, Transmitted by rodents and Hemorrhagic fever with renal failure** *Ref. Ananthanarayan 8/e 522, 9/e, p 527*

Already explained

51. Ans. (a, b, d, e) Zoonosis, Develop petechial rashes, Recently disease has been reported in Gujarat, It has high fatality

Ref: Ananthanarayan 8/e, p521-22, Harrison 18/e, p 1630

Crimean-Congo Hemorrhagic Fever/CCHF)

- Tick-borne viral disease, a zoonosis of domestic animals and wild animals, that may affect humans. Usually seen in East and West Africa, is a member of the Bunyaviridae family of RNA viruses.
- However, in ***January 2011***, out break occured in ***Gujarat*** that led to few deaths.
- Human infection is acquired via a tick bite or during the crushing of infected ticks. Domestic animals do not become ill, but do develop viremia.
- Clinical disease is rare in infected mammals, but commonly ***severe in infected humans, with a 30% mortality rate.***
- Though similar to other HF syndromes, Crimean-Congo HF is associated with extensive live damage.
- Laboratory values indicate DIC and show elevations in AST. creatine phosphokinase, and bilirubin.
- Patients with fatal cases generally have more marked changes, even in the early days of illness, and also develop leucocytosis rather than leucopenia.
- Cattle sheep goat and other domesticated animal acts as natural reservoir.

Chapter Review

1. **Man is the only reservoir of:** [AIIMS 90]
   a. Rabies b. Influenzae
   c. Typhoid d. Japanese B encephalitis
   [Ref. Park 22/e, p 213]

2. **Epidemic hemorrhagic conjunctivtis is caused:** [UP 02]
   a. HSV b. HZV
   c. HIV d. Picorna virus
   [Ref. Ananthanarayansa 8/e, p 490, 9/e, p 491; Table 54.2]

   It is caused by enterovirus 70, which isJ a subtype of picorna virus.

3. **Negri Bodies are commonly seen in:** [AIIMS 90, 92; AI 88]
   a. Hippocampus b. Hypothalamus
   c. Mamillary bodies d. Cerebrum
   e. Pons
   [Ref. Ananthanarayan 8/e, p 529, 9/e, p 531]

4. **The congenital rubella syndrome:** [DNB 02]
   a. May be prevented by vaccination in early pregnancy
   b. Causes intrauterine growth retardation
   c. Causes cataracts
   d. Causes deafness only if acquired before 16 weeks of gestation
   [Ref. Park 22/e, p 142]

5. **In india, human infections have been reported dengue virus type:** [AP 91]
   a. Types I and 1 b. Types 1 and 3
   c. Types 2 and 4 d. Type 1 only
   e. All 4 types
   [Ref. Park 22/e, p 225]

   In India type I and type II serotypes are common

6. **KFD is transmitted by:** [AI 91]
   a. Fleas b. Mite
   c. Tick d. Mosquito
   [Ref. Ananthanarayan 8/e, p 520, 9/e, p 524]

   KFD infection is transmitted by the bite of ticks, principal being Haemapysalis spinigera.

7. **Which of the following is not common in India:** [AI 91]
   a. Japnese B encphalitis
   b. Lassa fever
   c. KFD
   d. Dengue
   [Ref. Park 22/e, p 259]

8. **Rubella vaccination is contra indicated in all except:**
   a. Patient on immunosuppressant [JIPMER 02]
   b. Girl with Leukemia
   c. Girls between 11-14 years
   d. Pregnancy
   [Ref. Park 22/e, p 143]

9. **Acute epidemic keratoconjuctivitis is caused by:**
   a. Herpesvirus b. Echo 51 [AI 91]
   c. Enterovirus 70 d. Enterovirus 72
   [Ref. Ananthanarayan 8/e, p 491, 9/e, p 491]

10. **The following is true of Rotavirus:** [AI 92]
    a. Easily grown in cell culture
    b. Double stranded DNA
    c. Terminal ileum villi destroyed
    d. Adult and old people account for 60% of infection
    [Ref. Harrison 19/e, p 1288, 18/e, p 1590]

11. **Pre-exposure cell culture vaccine used in Rabies**
    a. 3 doses b. 4 doses [UP 02; AI 90]
    c. 5 doses d. 6 doses
    [Ref. Park 22/e, p 255]

12. **Break bone fevers caused by which virus:** [Bihar 03]
    a. Variola b. Coxsackie
    c. Arbo d. Adenovirus
    [Ref. Anantharayan 8/e, p 519, 9/e, p 523]

13. **All are true of rabies except:** [AIIMS 92]
    a. 100% mortality
    b. Spreads from periphery
    c. Infects only the brain
    d. Prophylactic immunization of people at Risk
    [Ref. Ananthanarayan 8/e, p 528, 9/e, p 532]

14. **Diarrrhea in Rotavirus infection is due to:** [PGI 93]
    a. Increased intestinal hypermotility
    b. Decreased absorption by villi
    c. Increased Secretion by villi
    d. None of the above
    [Ref. Harrison 19/e, p 1288, 18/e, p 1591]

15. **Rabies virus infection, what is seen in histology:** [DNB 2013]
    a. Schik cells c. Asteriod bodies
    b. Negri bodies
    d. None
    [Ref. Ananthanarayan 9/e, p 533]

Answers 1. c. Typhoid 2. d. Picorna ... 3. a. Hippocampus 4. b. and c 5. e. All 4 types
6. c. Tick 7. b. Lassa ... 8. c. Girls .... 9. c. Enterovirus ... 10. c. Termi ...
11. a. 3 doses 12. c. Arbo 13. c. Infects ... 14. b. Decreased ... 15. b. Negri ...

16. Rabies diagnosis is done best by: [DNB 2013]
a. Brain biopsy b. Blood culture
c. Electron microscopy d. None
[Ref. Ananthanarayan 9/e, p 533; Harrison 19/e, p 1302]

- Detection of rabies virus RNA by RT-PCR is highly sensitive and specific so is direct fluoroscence antibody.
- RT-PCR can be performed on fresh saliva, CSF, skin and brain tissues.
- DFA require biopsy from brain or skin

17. Epidemic of polio is due to: [Kolkata 03]
a. Type I virus
b. Type II virus
c. Type III virus
d. Combine of type II and type III virus
[Ref. Park 22/e, p 185]

18. Recommended vaccines for rabies: [Kar 04]
a. DPT b. MMR
c. BCG d. HDCV
[Ref. Park 22/e, p 254]

19. About rabies true is: [SGPGI 05]
a. Vaccine causes lifelong immunity
b. Multiple strains are found
c. CNS infention occurs through viremia
d. Bullet-shaped nonenveloped, double stranded RNA virus
[Ref. Ananthanarayan 8/e, p 524, 9/e, p 532-534]

20. Which of the following is associated with acute hemorrhagic conjunctivitis? [Bihar 05]
a. Rhabdovirus b. Enterovrus
c. Calcivirus d. Echovirus
[Ref. Ananthanarayan 8/e, p 491, 9/e, p 491]

21. Enteroviruses cause: [Bihar 05]
a. A. hemorrhagic conjunctivitis
b. Ac. follicular conjunctivitis
c. Posterior follicular conjunctivitis
d. Epidermic kerato conjunctivitis
[Ref. Ananthnaryan 8/e, p 490, 9/e, p 491]

Acute hemorrhagic conjunctivitis is caused by enterovirus 70 and Coxsackie virus type A 24. Both these virus shows intratypic antigenic difference.

22. Break bone fever is caused by which virus: [Bihar 05]
a. Varioloa
b. Coxsackie
c. Dengue
d. Adenovirus
[Ref. Anathnarayan 8/e, p 519, 9/e, p 523]

23. Commonest cause of Bronchiolitis is: [UP 05]
a. RSV b. Adenovirus
c. Influenza d. Herpesvirus
[Ref. Ghai 6/e, p 352]

24. In which of the following virus is shed in stool:
a. Herpangina b. Influenza [UP 96]
c. Varicella d. Smallpox
[Ref. Ananthanarayan 8/e, p 489, 9/e, p 492]

Herpangina (vesicular pharyngitis) is a common clinical manifestation of Coxsackie Group A infection in children. It is a severe febrile pharyngitis with headache, vomiting and pain in abdomen. Like all other enterovirus it also spreads through fecoral route.

25. Which of the following is true regarding influenza:
a. It is caused by an enveloped DNA virus [MP 06]
b. Laboratory studies may show neutropenia early in the course of disease
c. Primary infectious pneumonia is less common than secondary bacteria pneumonia
d. Antiviral agents is given early prevents complications *[Ref. Harrison 19/e, p 1212, 18/e, p 1496]*

26. Virus lacking hemagglutinin and nuraminidase but have membrane fusion protein is: [Jharkhand 06]
a. RSV b. CMV
c. HSV d. Ebestein Barr virs
[Ref. Anantharayan 8/e, p 508, 9/e, p 510]

- RSV differs from other paramytovirus in not possessing hemagglutinin activity and neurominidase activity. So it is non-hemolytic. Its nucleocapsid diameter is also less than that of other paramycovirus

27. Negri bodies are seein in infections due to: [Kar 06]
a. Poliovirus b. Rabiesvirus
c. Herpesvirus d. Adenovirus
[Ref. Ananthanarayan 8/e, p 529, 9/e, p 531]

28. A vaccine for rabies was first developed by: [Kar 06]
a. Louis psteur b. Robert koch
c. Edward jenner d. Landsteiner
[Ref. Ananthanarayan 7/e, p 2, 9/e, p 532]

29. Coxsackie group A commonly causes: [TN 01]
a. Conjunctivitis b. Aseptic meningitis
c. Hepatitis d. Myocarditis
[Ref. Ananthanarayan 8/e, p 491, 9/e, p 491]

30. Which of the following viruses is composed of two distinct capsids enclosing the double stranded RNA?
a. Adenovirus b. Reovirus [Kar 00]
c. Herpes virus d. Myxovirus
[Ref. Jawetz 24/e, p 502]

Answers **16.** a. Brain biopsy **17.** a. and c **18.** d. HDCV **19.** b. Multiple ... **20.** b. Enterovrus
21. a. A. hemorrhagic **22.** c. Dengue **23.** a. RSV **24.** a. Herpangina **25.** c. Primary ...
26. a. RSV **27.** b. Rabies ... **28.** a. Louis ... **29.** b. Aseptic... **30.** b. Reovirus

31. The vector for Japanese encephalitis is: [Kar 00]
a. Lice b. Tick
c. Culex mosquito d. Sandfly
[Ref. Park 22/e, p 259]

32. Type of vaccine available commercially for rabies are all except: [UP 00]
a. Inactivated sheep brain vaccine
b. Genetically engineered glycoprotein vaccine
c. Duck embryo cultured vaccine
d. Human diploid cell vaccine *[Ref. Park 19/e, p 229]*

33. All enveloped helical RNA viruses belong to one large group, which includes all of the following except:
a. Influenza b. Parainfluenza [Kar 01]
c. Mumps d. Herpes
[Ref. Ananthanarayana 8/e, p 494, 9/e, p 496]

Influenza, Para-influenza, mumps all belongs to myxovirus

34. Rotaviruses are responsible for: [Kar 01]
a. Acute nonbacterial gastroenteritis in adults
b. Infantile diarrhea
c. Teratogenic effects
d. Respiratory tract infection in immunocompromised individuals *[Ref. Harrison 19/e, p 1288, 18/e 1590]*

35. Acute hemorrhagic conjunctivitis is caused by: [AI 97]
a. Enterovirus b. Adenovirus
c. Poliovirus d. Hepadna virus
[Ref. AA 8/e p 490, 9/e p 491]

Acute epidemic hemorrhagic conjunctivitis or apollo conjunctivitis is caused by:
- Pneumococci
- Adeno virus
- Coxsackie virus type A - 24
- Enterovirus type 70 *(MC)* ***Mnemonic - PACE***

36. Reverse transcriptase polymerase chain reaction can aid in diagnosis of all of the following viral infection except: [AI 97]
a. Adenovirus b. Astrovirus
c. Rotavirus d. Poliovirus

RT - PCR is useful for RNA virus

37. All of the viruses cause pneumonia except: [AI 95]
a. Cytomegalovirus b. Mumps
c. Measles d. Retrovirus
[Ref. Harrison 18/e, p 1609]

38. Rotavirus is diagnosed by: [AIIMS 99]
a. IgM specific antibody in stool
b. ELISA demonstrates antibody in stool
c. Immunoflorescence antigen in stool
d. Culture of rotavirus *[Ref. AA 8/e, p 557, 9/e, p 562]*

39. Segmented double stranded RNA virus is seen in: [AIIMS 98; PGI 97]
a. Reovirus b. Myxovirus
c. Rabies d. Parvo virus
[Ref. AA 9/e, p 560]

40. Conjunctivitis is caused by all except: [AIIMS 98]
a. CMV b. Enterovirus 70
c. Coxsackie A 24 d. Adenovirus
[Ref. AA 8/e p 481, 9/e, p 491]

41. All are true about rotavirus except: [AIIMS 97]
a. Causes diarrhea in man and children
b. Rota B can be grown in cell culture
c. Rota C can cause diarrhea in children
d. Culture cannot be done *[Ref. AA 8/e p 557, 9/e, p 561]*

42. All are cultivable virus except: [AIIMS 97]
a. Rotavirus b. Enterovirus
c. ECHO virus d. Coxsackie virus
[Ref. AA 8/e p 551, 9/e, p 561]

Non-cultivable viruses:
- Rotavirus (certain strains can grow in tissue culture)
- Norwalk virus
- Mollascum contagiosum.

43. Lipid envelope is found in which virus? [PGI 98]
a. Reo
b. Herpes
c. Picorna
d. All of the above *[Ref. AA 8/e p 439, 9/e, p 466]*

Enveloped DNA viruses:
- Herpesviridae
- Hepadnaviridae (HBV)

All RNA viruses are enveloped except **PARC** (Picorna, Astro, Reo, Calciviridae).

44. All of the following rabies vaccines are commercially available except: [AI 99]
a. Killed sheep brain vaccine
b. Human diploid cell vaccine
c. Vero continuous cell vaccine
d. Recombinant glycoprotein *[Ref. Park 22/e p 254]*

45. True statement about rabies is: [AI 97]
a. Rabies infection causes life-long immunity
b. Rabies vaccine is always live attenuated
c. Rabies has only one strain
d. Rabies is best diagnosed by immuno-florescence study *[Ref. AA 7/e p 539, 9/e, p 533]*

Answers 31. c. Culex... 32. b. Genetically ... 33. d. Herpes 34. b. Infantile ... 35. a. Enterovirus
36. a. Adenovirus... 37. b. Mumps ... 38. c. Immuno ... 39. a. Reovirus ... 40. a. CMV
41. d. Culture... 42. a. Rotavirus ... 43. b. Herpes 44. d. Recombinant 45. d. Rabies

46. Negri bodes are found in: **[AI 96]**
a. Hypothalamus b. Hippocampus
c. Midbrain d. Medulla
[Ref. Ananthanarayan 9/e, p 539]

47. Rabies virus inactivated by: **[PGI 97]**
a. Phenol
b. UV radiation
c. BPL (Beta propiolactone)
d. All *[Ref. AA 8/e p 525, 9/e, p 530]*

- **Inactivating agent of rabies virus:**
  - Phenol
  - Formalin
  - Betapropiolactone
  - UV irradiaction
  - Sunlight
- Thermal inactivation occurs in one hour at 50°C and 5 min at 60°C.

48. Negri bodies are characteristic of viral infection by: **[PGI 97]**
a. Rabies
b. Toxoplasmosis
c. Polio *[Ref. Ananthanarayan 9/e, p 534]*
d. Herpes simpex infection

49. Hantan virus: **[PGI 96]**
a. Is a DNA virus
b. Causes hemorrhagic fever with renal involvement
c. Belong to Retroviridae family
d. Person to person transmission *[Ref. AA 9/e p 527]*

50. Antirabies vaccine is prepared by: **[DNB 2012]**
a. Street virus b. Fixed virus
c. Line virus d. Wild virus
[Ref. AA 8/e p 531, 9/e p 531]

51. Swine flu is caused by: **[DNB 2012]**
a. H_1N_1 b. H_5N_1
c. C_3N_1 d. H_3N_3
[Ref. Greenwood 18/e p 500]

52. Hand foot mouth diseases caused by: **[DNB 2013]**
a. Cocksackie A b. EBV
c. CME d. HSV
[Ref. Ananthanarayana 9/e, p 491]

Answers **46.** b. Hippocampus **47.** d. All **48.** a. Rabies **49.** b. Causes... **50.** b. Fixed virus **51.** a. H_1N_1 **52.** a. Cocksackie A

NEET Pattern Questions

1. Which is enveloped virus:
a. Dengue virus
b. Norwalk virus
c. Hep A virus
d. Adenovirus
[Ref. Harrison 18/e, p 1433, 1434]

All RNA virus are enveloped except Piorna, Calciveridaeg, Rioviridae, Astrovirus

2. Non-enveloped ss-RNA virus is:
a. Picronavirus
b. Poxvirus
c. Retrovirus
d. Bunyavirus *[Ref. Ananthanarayan, 9/e, p 484]*

3. Influenza virus culture is done on:
a. Charioallantoic membrane
b. Allantoic cavity
c. Yolk sac
d. All
[Ref. Ananthanarayan, 9/e, p 500]

Isolation of Influenza Virus
- Specimen : Throat garglings
- Tissue : Isolation can be made in eggs or in monkey kidney cell culture. For primary isolation the most suitable cells are Madin-Darby Canine Kidney (MDCK) cells.
- Procedure : The material is inoculated into the amniotic cavity of 11-13 day old eggs. After inoculation at 35°C for three days the eggs are chilled and the amniotic and allantoic fluids harvested separately. The fluid are then tested for hemagglutination separately.

Answers **1.** a. Dengue virus **2.** a. Picronavirus **3.** b. Allantoic cavity

4. **Most virulent dengue fever strain is:**
   a. 1 b. 2
   c. 3 d. 4

5. **Causative organism of SARS:**
   a. H1N1 b. Corona virus
   c. Rotavirus d. RSV
   [Ref. Harrison, 18/e, p 1487]

SARS is caused by corona virus type 4. It is characterized by pneumonitis and diarrhea.

6. **Antigenic shift:**
   a. Occurs every 2-3 years
   b. Gradual change over time
   c. Result from genetic recombination
   d. Occurs in all influenza viruses
   [Ref. Harrison, 18/e, p 1492]

Antigenic shift occurs every 10–15 years, whereas antigenic drift occurs every 2–3 years in case of influenza A and every 4–7 years in case of influenza B.

7. **Coxsackie virus is:**
   a. Herpes virus b. Pox virus
   c. Enterovirus d. Myxovirus
   [Ref. Ananthanarayan, 9/e, p 485]

8. **Herpangina is caused by:**
   a. Enterovirus b. Rhinoviruses
   c. Myxovirus d. Rabies virus
   [Ref. Ananthanarayan, 9/e, p 485]

Herpangina is caused by coxsachie virus group A which is a subtype of enterovirus.

9. **False about Polio:**
   a. Descending Paralysis
   b. Bilateral symmetrical
   c. Non-progressive
   d. LMN type paralysis
   [Ref. Ananthanarayan, 9/e, p 486]

10. **Diagnosis of polio:**
    a. Detection of polio virus in stool
    b. Serology
    c. Limb wasting
    d. AFP
    [Ref. Ananthanarayan, 9/e, p 487]

Isolation of virus in tissue culture is the best method for diagnosis. Virus can be isolation from blood (1-2 weeks after infection) from throat in early stages of disease, from feces in over 80% of cases in 1st week.

11. **Appearance of cowdry type A inclusion bodies:**
    a. Granular b. Circumscribed
    c. In polio d. None
    [Ref. Ananthanarayan, 9/e, p 445]

Cowdry classify intranuclear antibodies into two types:
(a) Cowdry type A: Variable size and granular in appearance
(b) Cowdry type B: More circumscribed and often multiple e.g. adenovirus and poliovirus

12. **Hemorrhagic fervr is caused by:**
    a. West-Mile fever b. Sandfly fever
    c. Ebola virus d. All of the above
    [Ref. Harrison, 16/e, p 1629]

13. **Suckling mice is used for isolation of:**
    a. Coxsachie Virus b. Pox
    c. Herpes d. Adeno Virus
    [Ref. Ananthanarayan, 9/e, p 491]

14. **HTLV extra gene is:**
    a. Gag b. Pol
    c. Env d. Tex
    [Ref. Ananthanarayan, 9/e p 567]

15. **Classic triad of congenital rubella includes all except:**
    a. Cataract b. Deafness
    c. Retinitis d. CHD
    [Ref. Ananthanarayan, 9/e, p 555]

Classic triad of congenital rubella:
- Cardiac cardiac defects
- Cataract
- Deafness

16. **All oncogenic viruses containing RNA belongs to:**
    a. Picornaviridae b. Herpesviridae
    c. Retroviridae d. Flaviviridae
    [Ref. Ananthanarayan, 9/e, p 565]

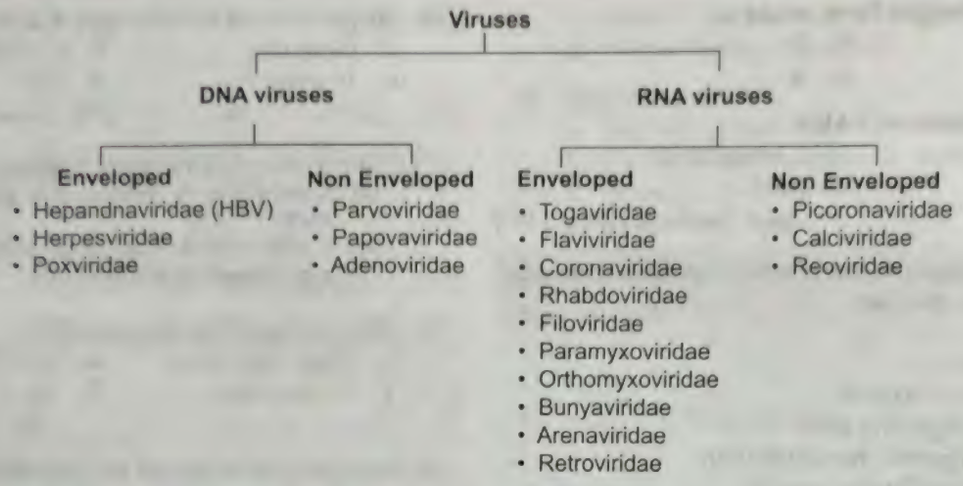
17. **Most common oncogenic RNA virus**
    a. Retrovirus b. Picornavirus
    c. Orthomyxovirus d. Paramyxovirus
    [Ref. Ananthanarayan, 9/e p 566]

18. **Colorado Tic fever is caused by:**
    a. Filoviridae b. Reoviridae
    c. Coronaviridae d. Calciviridae
    [Ref. Ananthanarayan, 9/e p 560]

19. **Which is enveloped virus?**
    a. Dengue Virus b. Norwalk Virus
    c. Hep A Virus d. Adeno Virus

Colorado tick fever is caused by orbivirus which is one of the three genera of reoviridae: Reovirus orbivirus and rotavirus

Answers
4. **b.** 2
5. **b.** Corona virus
6. **c.** Result from...
7. **c.** Enterovirus
8. **a.** Enterovirus
9. **b.** Bilateral...
10. **a.** Detection...
11. **a.** Granular
12. **c.** Ebola virus
13. **a.** Coxsachie...
14. **d.** Tex
15. **c.** Retinitis
16. **c.** Retroviridae
17. **a.** Rota virus
18. **b.** Reoviridae
19. **a.** Dengue virus



20. Hemagglutination done by all virus except:
a. Influenza b. Rubella
c. Measles d. HPV
[Ref. Ananthanarayan, 9/e p 431]

Virus causing hemogglulination
Influenza, Prainfluenza, Mumps, NDV, Measles, Togavirus, Rubella, Enterovirus, Cosacke & Echo, Rhinovirus, Rabies.

21. Double stranded RNA virus with segmented genome:
a. Influenza b. Rotavirus
c. Arenavirus d. Bunvavirus

Double stranded RNA virus includes rotavirus, reovirus, orbivirus all belongs to family reoviridae.

22. True about Nipah virus are all except:
a. Is a paramyxovirus
b. Causes hemorrhagic fever
c. Emerging infection
d. Present in India *[Ref. Jawetz, 27/e p 594]*

Nipah virus
- Zoonotic paramyxovirus that caused outbreaks of severe encephalitis (mortality rate >35%) in malaysia during 1998.
- Fruit bats are the natural host.

Note: Hendra virus (an equine virus) is another emerging zoonotic paramyxovirus that caused many horse fatalities and few human fatalities in Australia.

23. Which of the following is a RNA virus:
a. Herpes virus b. Adenovirus
c. Poxvirus d. Picornavirus

24. Acute hemorrhagic conjunctivitis occurs due to:
a. Poliovirus b. Hepadnavirus
c. Enterovirus 70 d. Coxackie B virus
[Ref. Ananthanarayan, 9/e p 492]

25. Paramyxoviruses enters the body via:
a. Blood b. Respiratory virus
c. Conjunctiva d. Fecal-oral route
[Ref. Ananthanarayan, 9/e p 507]

26. Double tranded RNA virus:
a. Rota virus b. Measles virus
c. Mumps virus d. Influenza virus

27. Virus which has more than one serotypes:
a. Measles b. Mumps
c. Rubella d. Influenza
[Ref. Ananthanarayan, 9/e p 499]

28. Diagnosis of Dengue rever can be made earlies by:
a. Viral culture
b. NS-1 antigen detection
c. IgG antibody detection
d. Nucleic acid test *[Ref. Jawetz, 27/e p 553]*

29. Influenza virus belongs to family:
a. Picornaviridae b. Calciviridae
c. Orthomyxoviridae d. Paramyxoviridae
[Ref. Ananthanarayan, 9/e p 499]

30. Hand-foot-mouth disease is caused by:
a. Coxsackie - A virus b. Coxsackie - B virus
c. EBV d. CMV

Coxsackeivirus A16 is the most common cause of HFMD. Enterovirus 71 (EV-71) is the second-most common cause.

31. Incubation period of measles is:
a. 18-72 hours
b. 10-14 days
c. 3-4 days
d. 20-25 days
[Ref. Ananthanarayan, 9/e, p 512]

Answers **20.** d. HPV **21.** b. Rotavirus **22.** d. Present in India **23.** d. Picornavirus
24. c. Enterovirus 70 **25.** b. Respiratory... **26.** a. Rotavirus **27.** d. Influenza
28. d. Nucleic acid test **29.** c. Orthomyxoviridae **30.** a. Coxsackie - A virus **31.** b. 10-14 days

32. Which of the following is live attenuated vaccine:
a. Salk polio vaccine b. Sabin polio vaccine
c. Rabies vaccine d. KFD vaccine
[Ref. Ananthanarayan, 9/e, p 488]

33. Forschheimer spot is seen in: *[Ref. Park, 22/e, p 142]*
a. Measles b. Chicken pox
c. Erythema infectiosum d. Rubella

Forschheimer spot are a fleeting enanthem seen as small, red spot (petechiae) on the soft palate in 20% of patients with rubella. They preceed or accompany the skin rash of rubella. They are not pathognomonic to rubella as similar spots can be seen in measles and scarlet fever

34. Hemagglutination done by all virus except:
a. influenza b. Rubella
c. Measles d. HPV
[Ref. Ananthanarayan, 9/e, p 431]

Hemagglutination is shown ly influenza virus, mumps, parain-fluenza, measles, Togavirus, rubella, enterovirus, coxsackie, ECHO, rabies, reovirus

35. Not true about paramyxoviruses:
a. Belong to family myxoviridae
b. Are DNA viruses
c. Have linear nucleic acid
d. Antigenically stable *[Ref. Ananthanarayan, 9/e, p 506]*

36. True about rotavirus: *[Ref. Harrison, 19/e, p 1288-89]*
a. Is a DNA virus
b. Has lipid envelop
c. Older vaccine cause intussusception
d. All are correct

37. Diagnosis of Dengue fever can be made earliest by:
a. Viral culture b. NS-1 antigen detection
c. IgG antibody detection
d. Nucleic acid test

For details c answers 37 b in explanatory answers.

38. Which of the following can infect ovary:
a. Mumps virus b. EBV
c. CMV d. Measles virus
[Ref. Ananthanarayan, 9/e, p 508]

39. Which influenzae strain, not of human origin and can cause pandemic: *[Ref. Park, 22/e, p 147]*
a. $H_1 N_1$ b. $H_2 N_2$
c. $H_5 N_1$ d. $H_9 N_1$

$H_5 N_1$ (causative agent of avian influenzae) is a strain with pandemic potential. It has got extremly high mortality.

40. True about influenza vaccine: *[Ref. Park, 22/e, p 146]*
a. Live vaccine is used most commonly
b. Live vaccine is given by nasal drops
c. Killed vaccine is given intramuscular in deltoid
d. All are correct

41. Paramyxoviruses enters the body via:
a. Blood b. Respiratory route
c. Conjunctiva d. Fecal-oral route
[Ref. Greenwood, 18/e, p 511]

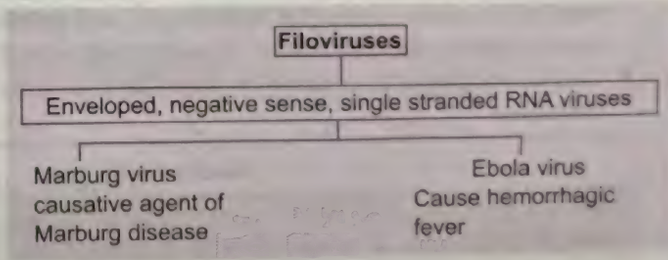
42. Influenza virus belongs to family:
a. Picornaviridae b. Calciviridae
c. Orthomyxoviridae d. Paramyxoviridae
[Ref. Ananthanarayan, 9/e, p 497]

43. Hand-food-mouth disease is caused by:
a. Coxsackie-A virus b. Coxsackie-B virus
c. FBV d. CMV
[Ref. Ananthanarayan, 9/e, p 491]

Hand, foot and mouth disease (HFMD)
- Exanthematous fever affecting mainly young children, characterized by clusters of papulovesicular lesions on the skin and oral mucosa.
- Coxsackie A-16, A9 and B1-3 were common causative agents initially.

44. True about rotavirus vaccine:
a. Killed vaccine *[Ref. Greenwood, 18/e, p 565]*
b. Given subcutaneous
c. Pentavalent vaccine
d. Should be given before 5 years

45. Ebola virus belongs to: *[Ref. Ananthanarayan, 9/e, p 559]*
a. Picornaviridae b. Togaviridae
c. Flaviviridae d. Filoviridae



46. Human metapneumovirus is structurally similar to:
a. Influenza virus b. Respiratory syncytial virus
c. Measles virus d. Rubella virus
[Ref. Ananthanarayan, 9/e, p 514]

47. Note true about Japanese encephalitis
a. Transmitted by culex mosquito
b. Caused by group A arbovirus
c. Pig is amplifier host
d. Man is incidental host *[Ref. Park, 22/e, p 259]*

Answers **32.** b. Sabin polio vaccine **33.** d. Rubella **34.** d. HPV **35.** b. Are DNA viruses **36.** d. Older vaccine cause intussusception **37.** d. Nucleic acid test **38.** a. Mumps virus **39.** c. H5 N_1 **40.** d. All are correct **41.** b. Respiratory route **42.** c. Orthomyxoviridae **43.** a. Coxsackie - A virus **44.** c. Pentavalent vaccine **45.** d. Filoviridae **46.** b. Respiratory syncytial virus **47.** b. Caused by group A arbovirus

CHAPTER

26

Slow Virus Disease

SLOW VIRUS INFECTIONS

They have the Following Characteristics:

- Incubation periods range from months to year.
- Course of illness lasting for months or years with remissions and exacerbations.
- Predilection for central nervous system.
- Absence of immune response or an immune response that does not arrest the disease but may actually contribute to pathogenesis.
- Genetic predisposition.
- Invariable fatal termination.

Remember: MC prion disorder in humans is sporadic form of CJD.

I

Slow Virus Disease:
- Fatal
- Altered immune response
- Very long incubation period
- MC slow virus disease: CJD

CLASSIFICATION

I. ***Group A***

- Slowly progressive infections of sheep caused by non-oncogenic retroviruses, lentiviruses – e.g. visna, maedi

I

Visna: Demyelinating disease of sheep characterized by slow onset paresis that progress to coma

II. ***Group B*** = *Subacute Spongioform viral encephalopathies*

- Comprise prion (infectious protein) diseases of the CNS, scarpie, mink encephalopathy, Kuru and CJD disease collectively known as the subacute spongiform viral emcephalopathies.
- These are chronic progressive degenerative diseases of CNS.
- Pathology consists of progressive vacuolation in the dendritic and axonal process of neurons and extensive astroglial hypertrophy and proliferations which leads to spongiform degeneration in the grey matter. There is no sign of any inflammation or immune response.
- Definative diagnosis is made by brain biopsy. After biopsy all instruments should be destroyed

Mechanism

PRPc is a protein encoded by PR Pgene located on chromosome 20. The protein is thought to play a role in synaptic function. In prion disease, there is misfolding of PRPc protein which is called as PRPsc protein

Normal individual
- PRPc protein
  - protein rich in α-helix less β sheets
  - Completely hydrolyzed by protease

↓

No accumulation

Patient with prion disease
- **PRPsc protein**
  - Primary structure same as PRP
  - More β sheets, little α helix
  - Resistance to protease

PRPc → PRPsc (Can convert normal to abnormal)

PRPsc → Accumulation → Formation of amyloid plaques

–PRPsc is not simply a misfolded protein but it is an alternatively folded molecule with a function.

I

- Subacute sclerosing panencephalitis: Caused by Measles, rubella virus
- Progressive multifocal leukoencephalopathy: Caused by JC virus.

Disease	Etiology
Human	
a. Kuru (meaning tremor)	Infection through ritualistic cannibalism
b. Iatrogenic Creutzfeldt-Jakob disease (CJD)	Infection from prion contaminated human growth hormone, duramater graft, corneal transplant
c. Variant (CJD)	Infection from bovine prion (Eating BSE infected beef)
d. Familial CJD	Germline mutation in PRNP
e. Sporadic CJD	Somatic mutation or spontaneous conversion of PRPc into PRPsc
f. Fatal familial insomania	Germline mutation in PRNP
g. Gestmann Strausster Scheinker	Germline mutation in PRNP
Animal prion disease, e.g.	
a. Scrapie	
b. Mink encephalopathy	
c. Bovine spongiform encephalopathy (BSE, mad-cow disease).	

III. *Group C*

Includes two unrelated CNS diseas

a. Subacute sclerosing panencephalitis (SSPE)
   - It is delayed sequel to infection with defective measles virus, characterized by slow progressive demyelination in CNS
   - Virus cannot be isolated in routine culture but only by co-cultivation of infected brain cells with susceptible cells of non-neural origin.
     - Antibody is regularly found in CSF and is **pathognomonic.**
   - ***SSPE also seen in Rubella infection.***

b. Progressive multifocal leukoencephalopathy (PML)
   - Seen in elderly persons whose immune proces is impaired by malignancy or HIV, etc.
   - Caused by JC virus (papovavirus).

Remember: JC virus also cause Hodgkin's disease of brain.

I
- SSPE & PML belongs to group C prion disease
- Kuru is transmitted due to cannibalism

Prions

- Subviral protein particles that are resistant to:
  - Heat
  - Ionizing and UV radiation
  - DNase & RNase
  - Formaldehyde and glutaraldehyde

Multiple Choice Questions

1. **Prions are:** [AI 08]
   a. Infectious proteins
   b. Made up of bacteria and virus particles
   c. Nuclear material
   d. Can be cultured in cell free media

2. **Prions consist of:** [AIIMS 07]
   a. DNA and RNA
   b. DNA, RNA and proteins
   c. RNA and proteins
   d. Only proteins

3. **Which of the following is not prion associated disease:** [AIIMS 03]
   a. Scarpie
   b. Kuru
   c. Creutzfeldt-Jakob disease
   d. Alzheimer's disease

4. **True about Prion disease is all except:** [AIIMS 01]
   a. Myoclonus is seen in 10% of the patients
   b. Caused by infectious protein
   c. Brain biopsy is diagnostic
   d. Commonly manifests as dementia

5. **True about prion:** [AIIMS 08]
   a. Are virus coded
   b. Cause misfolding of protein
   c. Cleave protein
   d. Defect in synthesis of protein.

6. **Which of the following is correct about prions?** [AIIMS Nov 2012]
   a. Long incubation period
   b. Destroyed by autoclaving at 121°C
   c. Nucleic acid present
   d. Immunogenic.

Explanations and References with Illustrative Answers

1. **Ans. (a) Infectious protein** *Ref. Jawetz 27/e, p 615*

   Prions are infectious protein devoid of nucleic acid.
   - Prions are usually resistant to standard means of inactivation. They are resistant to treatment with formaldehyde, urea, dry heat, boiling. However, they are sensitive to phenol, and autoclaving.
   - Prions infect and propagate by refolding abnormally into a structure which is able to convert normal molecule of protein into the abnormally structure form.
   - Common feature of all prions is conversion of a host encoded sialoglycoprotein into a protease resistant from which then accumulates.

2. **Ans. (d) Only protein** *Ref. Jawetz 27/e, p 3*

   Already explained, refer Ans. 1

3. **Ans. (d) Alzheimer's disease** *Ref. Harrison 17/e, p 2647, 18/e, p 3443*

   Various prion disease are as follows:

• Kuru	• Creutzfeldt-Jakob disease (CJD)
• Fatal familial insomnia (FFI)	• Gerstmann-Straussler-Scheinker disease (GSS)
• Sporadic fatal insomnia (SFI)	• Scrapie
• Transmissible mink encephalopathy	• Bovine spongiform encephalopathy (mad cow disease)
• Chronic wasting disease	• Feline spongiform encephalopathy
• Exotic ungulate encephalopathy	

4. **Ans. (a) Myoclonus is seen in 10% of the patients** *Ref. Harrison 17/e, p 2646 - 2650, 18/e, p 3445*
   - Most patients with CJD exhibit myoclonus that appears at various times throughout the illness.
   - Prion is proteinaceous infectious particle that lacks nucleic acid and causes slow progressing disease.
   - *MC* human prion disease is sporadic CJD.
   - Clinical Features of CJD
     - Most patient present with deficits in higher cortical function which almost always progress to dementia.
     - 90% patient exhibit myoclonus which persist during sleep in comparison of other involuntary movements.
     - Also present with visual impairment or cerebellar gait, coordination deficit, extrapyramidal dysfunction, pyramidal signs, seizures.
   - Diagnosis
     - Constellation of dementia, myoclonus and peirodic electrical burst in an afebrile 60 years old patient generally indicates CJD.
     - ***Only specific diagnostic test for CJD is measurement of PRPsc***
     - In humans the diagnosis of CJD as established by brain biopsy if PRPsc is detected.
       - There is no abnoramlity on gross examination of brain.
       - Pathologic hallmarks are spongiforms degeneration (in cerebral cortex, putamen, etc.) and Astrocytic gliosis.
       - 10% of CJD patient have amyloid plaques.
     - Sequencing the PRNP gene.
     - CJ finding may be normal or show cortical atrophy.
     - CSF is nearly always normal but may show minimal protein elevation.

5. **Ans (b) Cause misfolding of protein** *Ref. Greenwood 18/e, p 608, Harrison 18/e, p 3443*
   - Prion is an infectious agent made up of protein. In the presence of prion protein, the normal PRPc protein is converted into an abnormally folded PRPsc protein (protein with more β sheets). ***This PRPc protein is resistant to proteolysis and get accumulated.*** The "alternatively folded" is a better term than misfolded, as the abnormal protein has got alternative function.

6. **Ans. (a) i.e. Long incubation period** *Ref. Greenwood 18/e 607; Harrison 18/e, p 3441*

   Prion are infective proteins, that are resistant to inactivation by:
   - Heat
   - Ionizing radiation
   - DNAase & RNAase
   - Formaldehyde and glutaraldehyde

   Prion disease are characterized by absent immune reaction, long incubation period (*usually around 10 years*).

Note: Creutz feldt Jacob disease is the most common prion disease.

Chapter Review

1. **Mad cow disease is due to:** **[Culcutta 2K, UPGMEE 97]**
   a. Slow virus b. *Mycoplasma*
   c. Bacteria d. Fungus
   [Ref. Ananthanarayan 8/e, p 553, 9/e, p 557]

2. **Human cannibalism is associated with:** **[UP 06]**
   a. Q fever
   b. Sleeping sickness
   c. Trachoma
   d. Kuru
   [Ref. Ananthnaranayan 8/e, p 553, 9/e, p 557]

3. **All are human prion disease except:** **[UP 09]**
   a. Chronic wasting disease
   b. Fatal familial insomnia
   c. Creutzfeldt-Jakob disease
   d. Kuru
   [Ref. Ananthnaranayan 8/e, p 553, 9/e, p 557]

4. **Which one of the following not a prion associated disease:** **[JIP MER 05]**
   a. Scarpie
   b. Kuru
   c. Creutzfeldt-Jakob disease
   d. Alzheimer's disease
   [Ref. Ananthnaranayan 8/e, p 553, 9/e, p 557]

5. **Fatal familial insomnia is associated with:** **[AI 99]**
   a. Prion disease
   b. Degenerative disease
   c. Neoplastic disease
   d. Vascular disease *[Ref. Harrison 18/e 218]*

6. **Creutzfeldt-Jakob disease is caused by:** **[PGI 99]**
   a. Prion b. JC virus
   c. Genetic factors d. Nutritional deficiency
   [Ref. Ananthanarayan 9/e, p 557]

7. **Subacute sclerosing panencephalitis is the delayed manifestation of:** **[DNB 11]**
   a. Influenzae b. Measles
   c. Mumps d. Polio
   [Ref. Ananthanarayan 8/e 553, 9/e, p 557]

8. **All of the following human disease is caused by Prion except:** **[NEET]**
   a. SSPE b. Visna
   c. CJD d. Kuru
   [Ref. Ananthanarayan 8/e 552, 9/e, p 556-557]

Visna is a slow viral disease of sheep (associated with lentivirus)

SSPE is a delayed sequelae of measles

Answers 1. a. Slow virus 2. d. Kuru 3. a. Chronic ... 4. d. Alzheimer's ... 5. a. Prion disease
6. a and c. Prion, Genetic factors 7. b Measles 8. a, b. SSPE, Visna

NEET Pattern Questions

1. **Which of the following infection agent lacks RNA:**
   a. Virus b. Staphylococci
   c. Prions d. Cryptococcus
   [Ref. Jawetz, 27/e, p 3, 615]

2. **All of the following are prion disease except:**
   a. KJD
   b. Subacute spongiform encephalothies
   c. Mink Encephalopathy
   d. Burkitt lymphoma *[Ref. Ananthanarayan, 557-559]*

3. **Which prion disease affect human:**
   a. Scrapie
   b. Madcow disease
   c. Kuru
   d. Bovine spongiform encephalopathy
   [Ref. Jawetz, 27/e, p 614]

4. **Which of the following is infectious protein:**
   a. Avidin b. Prion
   c. Tau protein d. None *[Ref. Jawetz, 27/e, p 3]*

Answers 1. **b.** Prions 2. **d.** Burkitt lymphoma 3. **c.** Kuru 4. **b.** Prion

CHAPTER

27

Hepatitits Virus

Comparative Features of Viral Hepatitis

Feature	HAV	HBV	HCV	HDV	HEV
Family	Picornavirus	Hepadnavirus	Flavivirus	Defective virus	Calcivirus/ Alphavirus
Incubation (days) mean 40	15-45, mean 30	30-180, mean 60-90	15-160, mean 50	90-180 mean 60-90	14-60,
Onset	Acute	Insidious or acute	Insidious	Insidious or acute	Acute
Transmission					
Fecal-oral	+++	–	–	–	+++
Percutaneous	Unusual	+++	+++	+++	–
Perinatal	–	+++	±	+	–
Sexual	±	++	±	++	–
Clinical					
Severity	Mild	Occasionally severe	Moderate	Occasionally severe	Mild
Fulminant	0.1%	0.1-1%	0.1%	5-20%	1-2%
Progression to chronicity	None	Occasional (1-10%) (90% of neonates)	Common	Common	None
Carrier	None	0.1-30%	1.5-3.2%	Variable	None
Cancer	None	+	+	±	None
Prognosis	Excellent	Worse with age, *debility*	Moderate	Acute: good Chronic: poor	Good
Prophylaxis	IG Inactivated vaccine	HBIG Recombinant vaccine	None	HBV vaccine None for HBV carriers	Unknown
Therapy	None	Interferon Lamivudine Adefovir	Pegylated interferon plus ribavirin	Interferon ±	None

I

HAV: Picorna
HBV: Hepadna
HCV: Flavivirus
HDV: Defective
HEV: Calcivirus

Other virus causing sporadic hepatitis

- CMV
- HSV
- Enterovirus
- EBV
- Rubella virus

Note: Morphologic lesion of all types of viral hepatitis are similar and consist of panlobular infiltration with mononuclear cells, hepatic cell necrosis, kupter cell hyperplasia, cholestatis Liver cell damage consist of hepatic cell degeneration, ballooning of cells, acidophillic degeneration of hepatocytes (forming *councilman or apoptic bodies*).

I

Hepatitis A
- Non-enveloped ssRNA virus
- Over all MC cause of hepatitis
- Transmitted by feco-oral route
- Grows only in cells of primate origin

HEPATITIS A

- ***Non-enveloped ssRNA virus*** belonging to picorna family.
- Originally called as enterovirus 72.
- Resistant to ether, acid and heat but sensitive to chlorination.
- ***Only*** hepatitis virus that can be cultivated *in vitro*.
- ***MC*** cause of acute hepatitis in children.
- ***Only*** viral hepatitis which can cause spiky fever.
- As the age increases chances of jaundice increases

Diagnosis

Detection of *IgM anti-HAV* antibody during late incubation period which reaches peak level in 2-3 *weeks*.

Treatment

Symptomatic no specific antiviral drug is given.

Prevention

- ***Formalin inactivated tissue culture vaccine*** is effective.
- Given IM, two doses at 6 month interval
- Vaccine should be given in age > 2 years.
- Provides immunity after 4 weeks of vaccination.
- Immunity last for approx 20 years.

HEPATITIS B (SERUM HEPATITIS)

Most widespread and important type of hepatitis virus.

- ***Ds DNA virus belonging to hepadnaviruses family.***
- ***Transmitted parenterally*** (sex > perinatal > blood transfusion). Virus is stable and capable of surviving for days on environmental surface. It can be destroyed by sodium hypochlorite or autoclaving.

> *I*
>
> DNA polymerase of HBV has both DNA dependent DNA polymerase and RNA dependent reverse transcriptase activities

Viral Proteins and Particles

Three particulate form can be seen by electron microscopy:

1. 22 nm spherical or filament form (***MC***). Represent *excess viral envelop protein.*
2. 42 nm double shelled spherical particles. Represent intact HBV virion. ***(dane particle)***
3. Smaller spherical or tubular particle. Represent hepatitis B surface antigen **(HBS Ag = Australia Antigen).**

HBV Genes and Gene Products

Genes	Regions	Gene products
S	S	Major protein (S)
	S + Pre-S2	Middle protein (M) HBs Ag
	S + Pre-S1 and S2	Large protein (L)
C	C	HbcAg
	C + Pre-C	HbeAg
P (Largest gene)		DNA polymerase
X		***HBx Ag (contributes to carcinogenesis)***

Mutants of HBV

Two types of mutant are found:

- ***Hbe Ag Negative phenotype (MC mutant):*** Results in severe chronic infection with detectable HBV DNA and anti-HBe Ag but HBe Ag is not detectable.
- ***Escape mutant:*** Due to change in HBs Ag. Vaccine is not effective against them.

> *I*
>
> **Hepatitis B**
> - Ds DNA virus
> - Transmitted parenterally, sexually, vertically
> - First virological marker detectable: HBsAg
> - Diagnostic marker of acute hepatitis B: IgM anti HBC
> - Marker of Infectivity: HBe Ag

Pathogenesis and Clinical Features

1. **Acute hepatitis**
   - Low grade fever, jaundice, splenomegaly and tender hepatomegaly is found in all acute viral hepatitis.
   - None of the hepatitis virus is known to be directly cytopathic to hepatocyte, it is immunologic response which cause cell injury.

2. **Chronic hepatitis**
   - Persistence of HBeAg beyond 3 months or Persistance of HBsAg beyond 6 months after acute hepatitis.
3. **Perinatal transmission**
   - Occurs primarily in infants born to ***HBSAg carrier mothers or mothers with acute hepatitis B*** during third trimester.
   - Likelihood of perinatal transmission of HBV **correlates with presence of HBeAg;** 90% of HBeAg positive mothers but only 10 to 15% of anti-HBe positive mother transmit infection.
   - Most infection are transmitted at the time of delivery and are not related to breastfeeding.
   - Mostly acute infection in neonate is clinically asymptomatic, but the child is very likely to become HBs Ag carrier (90%).
4. **Extrahepatic manifestation of HBV**
   - Serum sickness like syndrome
   - Glomerulonephritis
   - Polyarteritis nodosa
   - Essential mixed cryoglobulinemia.
5. **Carrier stage**
   - Carriers is more common in patients of:
     - Down's syndrome
     - Polyarteritis nodosa
     - Lepromatous leprosy
     - Chronic renal disease
     - Leukemia
     - I.V. drug users
     - Hodgkin's disease
   - ***Carriers of HBs Ag particularly those infected in infancy have high-risk of hepatocellular Ca.***

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Extrahepatic HBV manifestation:
- Serum sickness
- Glomerulonephritis
- Polyarteritis nodosa
- Cryoglobulinemia

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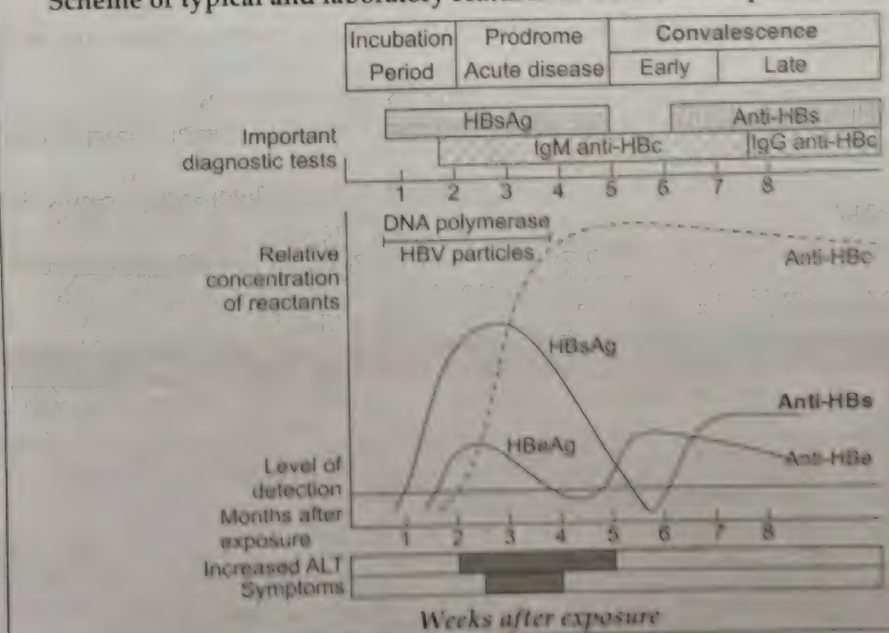
Carrier stage in HBV
- **Simple carriers**
  - HBs Ag +
  - HBV DNA –
  - Anti HBe –
- **Super carriers**
  - HBs Ag +
  - HBV DNA +
  - HBe Ag +
- **Super carriers are highly infective**

Serology and Diagnosis

A. **Serology**
   - ***First virologic marker*** detectable is HBsAg.
   - **Diagnostic *marker*** of ***acute*** hepatitis B infection IgM anti-HBc.
   - HBcAg is sequestered in HBsAg coat so, it is not routinely detectable.
   - Diagnosis in window period is made by AntiHBc.
   - **Marker of infectivity** - HBe Ag
   - *Titre of HBsAg bears inverse relationship with the degree of cell damage, i.e. titer is very low in acute fulminant hepatitis.*

Scheme of typical and laboratory features of acute viral hepatitis



- **Markers of replicative phase:**
  - HBeA : *Qualitative marker.*
  - HBV DNA : *Quantitative marker.*
- **Anti-HBSAg**
  - *Protective antibody*
  - Anti-HBSAg without anti-HBc **signifies vaccination.**
  - Anti-HBsAg in presence of IgG anti-HBc signifies recovery of infection.

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- Quantitative marker of replication: HBV DNA
- HBV vaccine: Non-glycosylated HBs Ag particles with alum as adjuvant

B. *Liver function test* (done in all case of acute hepatitis).
- ↑ ALT and AST (Level does not correlate with degree of cell damage).
- ↑ Bilirubin
- PT – **Prolonged value** signify hepatocellular necrosis and indicate bad prognosis.

Treatment

- Acute - Antiviral therapy is not used usually.
- In severe acute hepatitis B – Treatment with nucleoside analogue such as lamivudin can be given.

Prevention

- **Genetically engineered vaccine** from yeast consisting of nonglycosylated HBsAg particles.
  Dose: - 3 IM dose (in deltoid not in gluteal)
  - Injections are recommended at 0, I and 6 months.
  - *Pregnancy is not contraindication to vaccine.*
- ***Postexposure prophylaxis***: Combination of HBIG and hepatitis B vaccine simultaneously at different sites.
- ***Perinatal exposure:*** - *Single dose of HBIG 0.5 ml IM at birth followed by complete 3 dose of hepatitis B vaccine.*
  - First dose of vaccine should be given within 12 hours of life.

Remember: As hepatitis B can lead to hepatocellular carcinoma, vaccination makes ***HCC the only human cancer which is vaccine preventable.***

HEPATITIS D VIRUS = DELTA VIRUS

Defective virus that require helper function of HBV for its replication.
- Contains single stranded RNA and has replication cycle similar to some plant virus such as viroids or satellite virus.
- A new gene *deltavirus* has proposed for it, because of its special features.
- Delta core of HDV is encapsidated by an outer envelop of HBsAg, so it require cooperative function of HBV.
- Intracellular replication of HDV RNA can occur without HBV but liver injury require the presence of HBV.
- HDV antigen in liver and HDV RNA in serum and liver can be detected during HDV replication.
- HDV can cause either:

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Hepatitis D
- Defective delta virus
- ssRNA virus
- Can cause both superinfection, coinfection.

Features	Superinfection	Coinfection
Definition	• In patient already infected with HBV	• Infection simultaneously with HBV
Course	• Grave course	• Comparatively mild course
	• More chance of fulminant hepatitis and chronic infection	
Serology	IgM anti-HDV +	Ig M anti-HDV +
	IgM anti-HBc–ve	Ig M anti-HBC +ve

HEPATITIS C VIRUS

- Linear **SS RNA virus.**
- Belongs to gene hepacivirus of family flavivirus.
- ***MC* virus** associated with transfusion related hepatitis.
- **Cause fatty change in liver.**
- HCV is associated with ***Cryoglobinemia; porphyra cutania tarda; Idiopathic pulmonary fibrosis; membrane proliferative glomerulonephritis.***

Genome

- Envelope protein coded by hypervariable region varies from isolate-to-isolate and allow the virus to invade host immunity.
- Because of divergence of HCV isolates within a genotype or subtype and within the same host, these intragenotypic differences are referred to *as* quasispecies.

Serology and Diagnosis

- Assays of ***HCV RNA*** are the most sensitive test for HCV infection and represent the ***gold standard for diagnosis*** of hepatitis C (*HCV RNA detection has supplanted RIBA in most clinical settings).*
- Two types of Amplification techniques can be used to detect HCV RNA.
  - ***Branched chain complementary DNA (b DNA) assay.***
  - ***Reverse transcriptase PCR or TMA:*** It is more sensitive than b DNA assay.
- Anti-HCV antibody are not be detectable in acute phase and are not sensitive.
- **Anti-HCV are** not protective so chance of chronicity is very high.

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Presence of anti HCV in serum with out HCV RNA signifies recovery from HCV infection.

...CMDT 2014, p 650

Treatment

- In typical case of hepatitis C recovery is rare; *progression to chronic hepatitis is the rule.*
- Antiviral therapy with interferon alpha reduce the rate of chronicity.
- ***Duration of infection*** is the single most important variable which determine interferon responsiveness.

CHRONIC HEPATITIS C

Clinical features:
- *Fatigue* is *MC* symptom jaundice is rare.
- Extrahepatic manifestations may be seen.

Lab features:
- Aminotransferase fluctuate between high-to-high normal value.
- Both ALT and AST increase with ALT > AST.
- But when cirrhosis develop AST becomes greater than ALT
- ***LKM1 - Antibody may be seen in cases of HCV.***

Treatment:
- Combination therapy of pegylated interferon plus Ribavirin is now standard therapy.
- **Liver transplantation** - When cirrhosis develops.

I

Hepatitis C
- ssRNA virus belong to flavi virus family
- MC cause of transfusion hepatitis
- HCV RNA assay are the most sensitive test for diagnosis (RT PCR and b DNA assay)

Remember:
- Progression of liver disease is more likely in patients older age, longer duration of infection, advanced histologic stage and grade, HIV infection and obesity, increased hepatic iron.
- ***Chronic hepatitis C is MC indication*** of liver transplantation.
- ***Best prognostic*** indicator in chronic hepatitis C is liver histology.

..... Harrison, 19/e, p 2041

HEPATITIS E VIRUS

- Epidemic *hepatitis or enterically transmitted non-A, non-B hepatitis.*
- Non-enveloped *SS RNA* virus belonging to Hepeviredae family. *..... Jawetz, 27/e, p 498*
- Hepatitis of hepatitis *E. virus* is characteristically *associated with cholestasis.*
- *Most important* ***cause of fulminant hepatitis in pregnancy.***
- In India it is the most common form of acute hepatitis of **adults (Overall most common is Hepatitis A).**

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Hepatitis E: ssRNA virus causing fulminant hepatitis in pregnant female

HEPATITIS G VIRUS

- Blood-borne RNA virus.

Important points about chronic hepatitis			
Type of hepatitis	***Diagnostic test (s)***	***Autoantibodies***	***Treatment***
Chronic hepatitis B	HBsAg, IgG anti-HBc, HBeAg, HBV DNA	Uncommon	IFN-α, lamivudine
Chronic hepatitis C	Anti-HCV, HCV RNA	Anti-LKMI	PEG-IFN-α Plus ribavirin
Chronic hepatitis D	Anti-HDV, HDV RNA, HBsAg, IgG anti-HBc	Anti-LKM3	IFN-α

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Hepatitis G: Blood borne RNA virus

- **Other viral infections associated with hepatitis**
  - CMV
  - HSV
  - VZV
  - Measles
  - EBV
  - Lassa fever
  - Yellow fever
  - Marburg
  - Cox sackie virus
  - Enterovirus

Multiple Choice Questions

Hepatitis A

1. **Non-parenteral hepatitis is:** [AI 00, AIIMS 96]
   a. Hepatitis A b. Hepatitis B
   c. Hepatitis C d. Hepatitis D

2. **Which of the following hepatitis virus is cultivable:** [AIIMS 07]
   a. Hepatitis A b. Hepatitis B
   c. Hepatitis D d. Hepatitis E

Hepatitis B and D

3. **Serology of a young man shows HBsAg, however HBeAg is negative with normal levels of AST and ALT. He is asymptomatic. What is the next line of management?** [AI 08]
   a. Wait and watch b. Antivirus
   c. Immunoglobulins d. Liver transplant

4. **Which of the following hepatitis virus have significant perinatal transmission:** [AI 03]
   a. HEV b. HCV
   c. HBV d. HAV

5. **Reverse transcriptase of HBV is coded on following gene:** [AI 00]
   a. C gene b. S gene
   c. P gene d. X gene

6. **Marker for acute hepatitis B is:** [AIIMS 07]
   a. HBV DNA polymerase
   b. IgG anti HBc
   c. Core antgen (HbcAg)
   d. Anti-HbsAg

7. **In a patient of active chronic hepatitis B all are seen except:** [AIIMS 07]
   a. HbsAg b. IgM anti-HBcAg
   c. HbeAg d. Anti-HbsAg

8. **A 30 years old patient presented with history of jaundice for 10 days. His liver function tests showed bilirubin of 10 mg/dl, SGOT/SGPT - 1100/1450, serum alkaline phosphatase–240 IU. He was positive for HbsAg. What should be the confirmatory test to establish acute hepatitis B infection?** [AIIMS 06]
   a. IgM Anti-HBc antibody
   b. HbeAg
   c. HBV DNA by PCR
   d. Anti-HBc antibody

9. **A 30 years old lady delivered a healthy baby at 37 weeks of gestation. She was a known case of chronic hepatitis B infection. She was positive for HBsAg but negative for HBeAg. Which of the following is the most appropriate treatment for the baby?** [AIIMS 05]
   a. Both active and passive immunization soon after birth
   b. Passive immunization soon after birth and active immunization at 1 year of age
   c. Only passive immunization soon after birth
   d. Only active immunization soon after birth

10. **The best diagnostic test for recent hepatitis B is:** [AIIMS 03, 01, 00, PGI 99, 97]
    a. HBsAg b. IgM anti-HBcAg
    c. Anti-HBe d. Anti-HBs

11. **A 30 years man presented with nausea, fever and jaundice of 5 days duration. The biochemical tests revealed a bilirubin of 6.7 mg/dl (conjugated 5.0 mg/dl) with SGOT/SGPT (AST/ALT) of 1230/900 IU/ml. The serological tests showed presence of HBs Ag IgM anti-HBc and HBeAg. The most likely diagnosis:** [AIIMS-02]
    a. Chronic hepatitis B infection with high infectivity
    b. Acute hepatitis B infection with high infectivity
    c. Chronic hepatitis B infection with low infectivity
    d. Acute hepatitis B infection with low infectivity

12. **Which of the following hepatitis virus is a DNA virus:** [AIIMS 02]
    a. Hepatitis C virus b. Hepatitis B virus
    c. Delta agent d. Hepatitis E virus

13. **A mother is HBsAg positive at 32 weeks of pregnancy. What should be given to the newborn to prevent neonatal infection:** [AIIMS 02]
    a. Hepatitis B vaccine + immunoglobulins
    b. Immunoglobulins only
    c. Hepatitis B vaccine only
    d. Immunoglobulins followed by vaccine 1 month later

14. **Hepatitis B vaccination is given to a patient. His serum will reveal:** [AIIMS 02]
    a. HBsAg
    b. Anti-HBsAg
    c. IgM Anti-HBc Ag and HBS Ag
    d. IgM and IgG Anti-HBc Ag

15. **Acute hepatitis B can be earliest diagnosed by:** [AIIMS 01, 95]
    a. IgM anti-HBcab b. HBsAg
    c. IgG anti-HBcAb d. Anti-HBsAb

16. **Best epidemiological tool for investigation of hepatitis B is:** [AIIMS 00; AI 97]
    a. Anti-HBsAg b. Anti-HBcAg
    c. Anti-HBeAg d. HBcAg

17. **In a patient only anti-HBsAg is positive in serum all other viral markers are negative. This indicates:** [AIIMS 00]
    a. Acute hepatitis b. Chronic active
    c. Persistent carrier d. Hepatitis B vaccination

18. If a patient was immunized with hepatitis B vaccine, which of the following is seen in serum: [PGI 06]
a. HBeAg b. HBSAg
c. Anti-HBs antibody d. Anti-HBe antibody
e. Anti-HBc antibody

19. Which of these is not a marker of active replicative phase of chronic hepatitis B? [AIIMS 08]
a. HBV DNA b. HBV DNA polymerase
c. Anti-HBC d. AST and ALT

20a. About hepatitis B, false statement is:
a. Vertical transmission is more important than horizontal [AIIMS Nov 10, May 12]
b. Communicable period lasts for months
c. Virus can be found in blood 1 month before jaundice
d. Age of onset determines the prognosis

20b. Reverse transcriptase is a RNA dependent DNA polymerase. Which of these use it:
a. Hepatitis A virus
b. Hepatitis B virus
c. Hepatitis E virus
d. Hepatitis C virus

Hepatitis C

21. HCV is associated with: [AI 00]
a. Anti-LKM-1 antibody b. Scleroderma
c. Cryoglobulinemia d. Polyarteritis nodusa

22. Chronic liver disease is caused by: [AI 00]
a. Hepatitis B b. Hepatitis A
c. Hepatitis C d. Hepatitis E

23. Hepatitis C virus is a: [AIIMS 04, PGI 98]
a. Togavirus b. Flavivirus
c. Filovirus d. Retrovirus

24. Most common hepatotropic virus causing increased chronic carrier state is: [AIIMS 97, 01]
a. HEV
b. HAV
c. HBV
d. HCV

25. Hepatitis C virus true finding is: [AIIMS 98]
a. Spreads along feco-oral route
b. Antibody to HCV may not be seen in acute stage
c. Does not cause chronic hepatitis
d. It cannot be cultured

26. HCV is: [PGI 05]
a. Enveloped RNA virus
b. Unenveloped RNA virus
c. Unenveloped positive strand RNA
d. Unenveloped negative strand RNA
e. DNA virus

Hepatitis E

27. A young pregnant woman presents with fulminant hepatic failure. The most likely etiological agent is:
a. Hepatitis B virus b. Hepatitis C virus [AI 04]
c. Hepatitis E virus d. Hepatitis A virus

28. A pregnant woman from Bihar presents with hepatic encephalopathy. The likely diagnosis: [AI 01, 95]
a. Hepatitis E
b. Hepatitis B
c. Sepsis
d. Acute fatty liver of pregnancy

29. During epidemic of hepatitis E, fatality is maximum in: [DNB 08, AI 00]
a. Pregnant women
b. Infants
c. Malnourished male
d. Adolescents

30. With which of the following of viral hepatitis infection in pregnancy, the maternal mortality the highest:
a. Hepatitis A
b. Hepatitis B [AIIMS 06]
c. Hepatitis C
d. Hepatitis E

31. Which of the following is calcivirus: [AIIMS 01]
a. HEV
b. HBV
c. HCV
d. HAV

Miscellaneous

32. A blood donor is not considered for safe transfusion, if he has: [AI 00]
a. Anti-HBS Ab +ve
b. Anti-HBS Ab and HBc Ag +ve
c. HBs Ag +ve and IgM anti-HBc +ve
d. Anti-HBe +ve

Explanations and References with Illustrative Answers

1. **Ans. (a) Hepatitis A** *Ref. Harrison 19/e, p 2006, 18/e, p 2546*

Transmission	
Parenterally	***Feco-oral***
Hepatitis B	Hepatitis A
Hepatitis C	Hepatitis E
Hepatitis D	
Hepatitis G	

- MC hepatitis associated with blood transfusion is hepatitis C.

2. **Ans. (a) Hepatitis A** *Ref. Ananthanarayan 9/e, p 541*

HAV can be grown in some human and simian cell cultures and is the only human hepatitis virus which can be cultured in vitro. It has also been cloned.

Remember:
- HAV is the hepatitis virus – That causes spiky fever
  – Show no perinatal transmission

The risk of transmitting HAV is greatest from 2 weeks before to 1 week after the onset of Jaundice.

3. **Ans. (a) Wait and watch** *Ref. Harrison 19/e, p 2020, 18/e, p 2554*
   - In hepatitis B, among previously healthy adult who present with clinically apparent acute hepatitis recovery ocurs in 99% therefore, antiviral therapy is not likely to improve the rate of recovery and is not required.
   - In this case also patient is virtually asymptomatic, so no treatment is required.

4. **Ans. (c) HBV** *Ref. Harrison 19/e, p 2013, 18/e, p 1939*

Perinatal transmission of various hepatitis virus				
HAV	HBV	HCV	HDV	HEV
–	+++	+/–	+	–

- **HBV is *MC* hepatitis virus that is transmitted perinatally.**
- *Most important* **factor** that determine perinatal transmission is HBeAg (risk 90%).
- ***MC* time** of perinatal transmission is at the **time of delivery.**
- ***MC* presentation** in neonate is asymptomatic **HBsAg carrier.**
- *Most effective* **treatment** of neonatal infection is HBIg immediately after delivery followed by complete 3 dose immunization by HBsAg vaccine. First dose within first 12 hours of life.

5. **Ans. (c) P gene** *Ref. Ananthanarayan 8/e, p 540, 9/e, p 544*

HBV genes and gene products			
Genes	***Regions***	***Gene products***	
S	S	Major protein (S)	HBsAg
	S+Pre-S_2	Middle protein (M)	
	S+Pre-S_1 and S_2	Large protein (L)	
C	C	HbcAg	
	C+Pre-C	HbeAg	
P (Largest gene)		DNA polymerase	
X		HBxAg	

DNA polymerase of HBV has two activities:
- DNA dependent DNA polymerase.
- RNA dependent Reverse transcriptase.

6. **Ans. (a) HBV DNA polymerase** *Ref. Jawetz 27/e p 503*
   - DNA polymerase activity, HBV DNA and HBeAg which are representative of viremic stage of hepatitis B, appears early in the incubation period, concurrently or shortly after the first appearance of HBsAg.
   - Thus, from these lines it is clear that DNA polymerase is a marker of HBV infection. However, it is elevated in both acute and chronic active hepatitis. As there is no other correct option, this can be taken as correct.

Note: Hepatitis core antigen never appears in serum as it remain inside hepatocyte (intrahepatic)

7. **Ans. (b) IgM anti-HBcAg** *Ref. Harrison 19/e, p 2032, 18/e, p 2550*

IgM anti-HBcAg is seen in acute hepatitis not in chronic active.

Marker of chronic active hepatitis: HBsAg+, IgG anti-HBcAg, HBV DNA+, HBeAg+

There is confusion in *option d* (Anti-HbsAg) also:

Harrison 17/e, p 1943 writes: – 10-20% of person with chronic HBV infection may harbor low level Anti-HBs. This antibody is not directed against common variant a, but against heterotopic subtype determinant.

There can be two pattern of chronic hepatitis B in respect to HBeAg

HBe Ag reactive chronic hepatitis

Replicative phase
- HBV DNA +
- HBeAg +
- Intrahepatocyte HBcAg +
- DNA polymerase +

Non replicative phase
- HBeAg very low
- HBV DNA very low
- Anti-HBe +
- Intrahepatocyte HBcAg –ve

HBeAg negative chronic hepatitis
- HBeAg –ve
- HBV DNA +
- DNA polymerase +
- Most cases represent precore or core promotor mutation
- Though HBV DNA level is less, injury and progression to cirrhosis is common

8. **Ans. (a) IgM anti-HBc antibody** *Ref. Harrison 19/e, p 2007, 18/e, p 2551*

Types	Serological markers of HBV
Acute hepatitis B	IgM anti-HBcAg +ve ; HBsAg +ve
Chronic hepatitis B	Ig G anti-HBcAg +ve ; HBsAg +ve
Marker of vaccination	Anti-HBsAg (+) ve Alone
Marker of remote infection *(Used for epidemiological purposes)*	IgG anti-HBc
Marker of infectivity	*HBeAg* ***(If present indicate high infectivity)***

- Test diagnostic of acute infection – IgM anti-HBc
- Test diagnostic of chronic infection – IgG anti-HBc + HBsAg
- Test for determining infectivity – HBeAg.

9. **Ans. (a) Both active and passive immunization soon after birth** *Ref. Harrison 19/e, p 2013, 18/e, p 2547*

Perinatal Transmission of Hepatitis B
- Likelyhood of perinatal transmission of HBV *correlates* with ***presence of HBeAg.*** 90% of HBeAg +ve and only 10-15% of anti-HBe +ve mother transmit infection.
- Most infection are transmitted at the time of delivery.
- Acute infection in neonate is clinically asymptomatic but the child is likely to become HBsAg carrier and have high risk *of developing hepatocellular carcinoma.*

Treatment of perinatal exposure:

For all infant born to HBsAg positive mother:

- *A single dose of HBIG* should be given intramuscularly in thigh *soon after birth.*
- Followed by complete course of three injections of recombinant *hepatitis B vaccine* with first dose *to be given with in the first 12 hour of life.*
- Subsequent dose of active immunization should be given after 1 month and 6 months.

10. Ans. (b) IgM anti-HBcAg *Ref. Harrison, 19/e, p 2007, 18/e, p 2551*

Remember:

- **Best marker for diagnosing acute hepatitis B is IgM anti-HBc** as it is found only in acute phase of hepatitis B (In chronic hepatitis IgG anti-HBc is found).
- First marker after acute hepatitis B infection is HBsAg but as it can be found in chronic infection too so, it is not reliable of acute infection.

So, Guys be clear:

- **Diagnostic marker of acute hepatitis B:** IgM anti-HBc
- **Earliest marker of acute hepatitis B:** HBsAg.

11. Ans. (b) Acute hepatitis B infection with high infectivity *Ref. Harrison 19/e, p 2017, 18/e, p 2550*

See the following table, **Do not try to learn it, just try to understand.**

Commonly Encountered Serologic Patterns of Hepatitis B Infection

HBsAg	Anti-HBs	Anti-HBc	HBeAg	Anti-HBe	Interpretation
+	–	IgM	+	–	Acute hepatitis B, high infectivity
+	–	IgG	+	–	Chronic active Hepatitis B, high infectivity
+	–	IgG	–	+	1. Late acute or chronic hepatitis B, low infectivity 2. HBeAg-negative (precore mutant) hepatitis B
+	+	+	+/-	+/-	1. HBsAg of one subtype and heterotypic anti-HBs (common) 2. Process of seroconversion from HBsAg to anti-HBs (rare)
–	–	IgM	+/-	+/-	• Acute hepatitis B • Anti-HBc "window"
–	–	IgG	–	+/-	• Low-level hepatitis B carrier or • Hepatitis B in remote past
–	+	*IgG*	–	+/-	*Recovery from hepatitis B*
–	+	–	–	–	1. Immunization with HBsAg (after vaccination) 2. Hepatitis B in the remote past 3. False-positive

12. Ans. (b) Hepatitis B virus *Ref. Harrison 19/e, p 2006, 18/e, p 2539*

Virus	Morphology and genome	Family
HAV	Non-enveloped RNA SS$^+$	Picorna virus
HBV	DNA	Hepadna virus
HCV	Enveloped RNA SS$^+$	Flavivirus
HDV	Enveloped RNA SS$^-$	Defective virus
HEV	Nonenveloped RNA SS$^+$	Hepeviridae

$^+$ = Positive strand $^-$ = Negative strand

Mnemonic: **pH Fall Dangerous Hai**

13. Ans. (a) Hepatitis B vaccine + immunoglobulins *Ref. Park 22/e, p 196*

Simultaneous administration of HBIG and hepatitis B vaccine is more efficacious than HBIG alone; as HBIG does not interfere with the antibody response to HBV vaccine and provide immediate protection.

14. **Ans. (b) Anti-HbsAg** *Ref. Harrison 19/e, p 2013, 18/e, p 2550*

Already explained

15. **Ans. (b) HBsAg** *Ref. Harrison 19/e, p 2013, 18/e, p 2550*

Here question is about the earliest diagnostic marker, **which is no doubt HBsAg.**

- HBsAg in absence of IgM anti-HBc or IgG anti-HBc indicates early acute infection.

So, go with **HBsAg.**

Remember:
- Dignostic marker of Acute hepatitis B – IgM anti-HBC
- Earliest marker of Acute hepatitis B – HBsAg.
- Diagnosis of HBV infection is usually made by HBsAg (Australia antigen).

In cases where HBsAg is too low diagnosis can be established by the presence of IgM anti-HBc ...*Harrison 17/e, p 1942*

16. **Ans. (b) Anti-HBcAg** *Ref. Harrison 19/e, p 2017, 18/e, p 2550 (Table 304.5)*

Already explained

17. **Ans. (d) Hepatitis B vaccination** *Ref. Harrison, 19/e, p 2017, 18/e, p 2550*

Already explained

18. **Ans. (c) Anti-HBs antibody** *Ref. Harrison 19/e, p 2017, 18/e, p 2550*

Already explained

19. **Ans. (c) Anti-HBC** *Ref. Jawetz 24/e, p 475, Harrison 19/e, p 2017, 18/e, p 2550*

Anti-HBC has no relation with viral replication

- HBV DNA, HBV DNA polymerase represent active viral replication
- Level of HBV DNA correlates well with the degree of liver injury as suggested by raised AST, ALT
- It is not compulsory in chronic active hepatitis that HBeAg is positive. Infact HBeAg negative chronic hepatitis B is more dangerous.

20(a). **Ans. (a) Vertical transmission is more important than horizontal** *Ref. CMDT 2014/e, p 646; Park 22/e, p 193*

Epidemiology of HBV

- Endemic throughout the world
- Based on the carrier stage, countries can be divided into three categories:
  a. ***High Endemicity:*** (> 8%) Vertical transmission is more common
  b. ***Intermediate Endemicity:*** (2-8%) India belongs to this category. Vertical = Horizontal transmission
  c. ***Low Endemicity:*** (<2%) European countries Horizontal transmission is more common
- **Mode of transmission:** Inoculation of infected blood or blood products, sexual contacts, vertical (Mother to fetus). Overall greatest number of cases results from heterosexual transmission. In India blood transfusion related infections are equally common.
- **Period of communicability:** Virus is present in body secretions and blood during the incubation period (for a month before jaundice) and acute phase of disease.
  Period of communicability is usually several months (occasionally years in chronic carriers) or until disappearance of HBsAg.
- ***Outome*** of HBV infection is age dependent with chances of acute hepatitis is ***directly proportional to age*** and occurs in 1% of perinatal, 10% of early childhood and 30% of late (> 5 years age) HBV infection.
- Development of ***chronic*** HBV infection is ***inversely related to age*** and occurs in 95% of infants infected perinatally, 5-10% infected after 5 years of age.

Remember: Period of infectivity for HAV: 2 weeks before and 1 week after the onset of jaundice.

20(b). **Ans. (b) Hepatitis B virus**

See below: DNA polymerase of HBV has both DNA-dependent DNA polymerase and RNA-dependent reverse transcriptase activities.

Note: Instead of DNA replication directly from a DNA template, hepadnaviruses rely on reverse transcription (effected by the DNA polymerase) of minus-strand DNA from a "pregenomic" RNA intermediate. Then plus-strand DNA is transcribed from the minus strand DNA template by the DNA-dependent DNA polymerase and converted in the hepatocyte nucleus to a covalently closed circular DNA, which serves as a template for messenger RNA and pregenomic RNA.

21. Ans. (a) and (c) Anti-LKM-1 antibody and Cryoglobulinemia *Ref. CMDT 2014, p 650*

See, the following line.

"HCV is a pathogenic factor in cryoglobulinemia and membranoproliferative GN and may be related to lichen planus, Autoimmune thyroiditis; Lymphocytic sialedinitis; Idiopathic pulmonary fibrosis; Porphyria cutanea tarda; Monoclonal gammopathies and lymphoma, increase risk of Type II diabetes mellitus."....CMDT' 2014, p 650

Now *see Harrison 17/e, p 1963*

"Some patient with serologically confirmable chronic hepatitis C have circulating anti-LKM-1. The occurrence of anti-LKM may result from partial sequence homology between the epitope recognized by anti-LKM-1 and 2 segments of HCV polyprotein."

Note: Hepatic steatosis is a particular feature of infection with HCV genotype 3.

So, inspite of wasting time for 1 answer switch over to next question.

Remember:
- LKM 1 antibody – Hepatitis C
- LKM 2 antibody – Drug induced hepatitis
- LKM 3 antibody – Hepatitis D

22. Ans. (c) Hepatitis C *Ref. Harrison 19/e, p 2006, 18/e, p 2546*

Decreasing order of progression to chronicity

	HCV	> HDV	> HBV	> HAV = HEV
Frequency of Chronicity	70-80%	5-20%	1-10% **90% of neonates**	None (0.1%)

Remember:
- HCV is MC cause of chronic hepatitis.
- **Chronic hepatitis C** is **MC** indication of **liver transplantation.**

23. Ans. (b) Flavivirus *Ref. Harrison, 19/e, p 2010, 18/e, p 2539*

Already explained

HEV although resembling calcivirus appears to be sufficiently distinct from any known agent to merit a new classification of its own as a unique gene hepevirus with in the hepeviridae family. ..... *Harrison 19/e, p 2010*

So, if question comes on **HEV** *go with* **herpeviridae** *not with calcivirus.*

24. Ans. (d) HCV *Ref. Harrison 19/e, p 2014, 18/e, p 2553*

Already explained

25. Ans. (b) Antibody to HCV may not be seen in acute stage *Ref. Harrison, 17/e, p 1937, 1943, 18/e, p 2551; CMDT 2014 p 654*

Anti HCV can be detected is acute hepatitis during the initial phase of elevated aminotransferase activity. The antibody however may never become detectable in 5-10% of patients with acute hepatitis C; Level of anti HCV become undetectable after recovery (which is very rare).

- Assays for **HCV RNA** are most sensitive test for HCV infection and represent **gold standard** in diagnosis.

Note: In chronic hepatitis C, anti HCV is detectable in > 95% of cares.

Other options

- Only hepatitis virus that can be cultured *in vitro* is HAV. But other hepatitis virus can be cloned in *E. coli.*
- In ***option "d"*** it is not clear whether they are asking culturability *in vitro* or *in vivo* (clonability) so, this option may be partially correct.
- HCV is transmitted parenterally.

26. **Ans. (a) Enveloped RNA virus** *Ref. Harrison 19e, p 2014, 18/e, p 2539*

Already explained

27. **Ans. (c) Hepatitis E virus** *Ref. Harrison 17/e, p 1941; COGDT 9/e, p 439, 18/e, p 2540*

Learn the following characteristics:

HAV	Cause spiky fever
HBV	Only hepatitis virus which is DNA virus, may cause cytopathic effect
HCV	Cause fatty change
HDV	Defective virus
HEV	Cause fulminant hepatitis in pregnant woman; associated with cholestasis.

Remember: HEV
- SS RNA virus belonging to alpha virus family. Transmitted feco-orally.
- Secondary person-to-person transmission is rare (C/F to other enteric born infection).

28. **Ans. (a) Hepatitis E** *Ref. COGDT 9/e, p 439*

MC cause of ***fulminant hepatitis*** in pregnancy - ***Hepatitis E.***

MC cause of ***hepatic encephalopathy*** in pregnancy is - ***Hepatitis E.***

29. **Ans. (a) Pregnant women** *Ref. Jawetz p 25/e, p 477; COGDT 9/e, p 439*

Already explained

30. **Ans. (d) Hepatitis E** *Ref. Harrison 18/e, p 2552*

Already explained

31. **Ans. (a) HEV** *Ref. Ananthanarayan, 9/e, p 550*

According to *Ananthanarayan 9/e, p 550*

"HEV has been classified in genus hepesvirus under family calciviridae."

But according to *Harrison 18/e 2543*

"HEV although resembling calicivirus is sufficiently distinct from any known agent to merit a new classification of its own as a unique genus; Hepevirus with in the family Hepeviridae."

32. **Ans. (c) HBsAg +ve and IgM anti-HBc +ve** *Ref. Harrison 19/e, p 2017' 18/e 2551*

- **HBSAg +ve and IgM anti-HBc +ve suggest acute viral hepatitis.**
- So, transfusion from this donor is contraindicated.

Chapter Review

1. **Which of the following acute viral hepatitis infections has the highest risk of progression to chronicity?** [Kar 03]
   a. Hepatitis C b. Hepatitis B
   c. Hepatitis A d. Hepatitis E
   [Ref. Harrison 19/e, p 2040, 18/e, p 2551]

2. **Which hepatitis virus is notorious for causing a chronic hepatitis evolving cirrhosis?** [Kar 04]
   a. Hepatitis C virus b. Hepatitis B virus
   c. Hepatitis E virus d. Cytomegalovirus
   [Ref. Harrison 19/e, p 2040, 18/e, p 2578]

In 1/4th of cases chronic hepatitis C progress to cirrhosis

3. **A potent vaccine is available for:** [AI 90]
   a. Hepatitis A b. Hepatitis B
   c. Malaria d. Respiratory syncitial virus
   [Ref. Ananthanarayan 8/e, p 538, 544, 9/e, p 548]

4. **Hepatitis A is transmitted by:** [AI 90]
   a. blood route b. Inhalation
   c. Feco-oral route d. All
   [Ref. Harrison 19/e, p 2004, 18/e, p 2546]

5. **HAV is not destroyed by:** [AIIMS 91]
   a. 0.5 ppm chlorine
   b. 1:4000 formalin
   c. UV radiation
   d. Boiling at 100°C for 5 minutes
   [Ref. Ananthanarayan 8e, p 537, 9/e, p 541]

HAV is inactivated by boiling for one minute; 1:4000 fo maldehyde at 37°C for 72 hours and chlorine 1 ppm for 30 min. It is not affected by anionic detergents, ether

6. **Which of the hepatitis prevalent in pregnancy-** [UP 07]
   a. Hepatitis A b. Hepatitis B
   c. Hepatitis C d. Hepatitis E
   [Ref. Ananthanarayan 8/e, p 546, 9/e, p 550]

7. **The serological marker of acute hepatitis B infection is:** [AI 92]
   a. HBsAg + HBeAg
   b. HBsAg + Core antibody (IgM)
   c. HBsAg
   d. HBcAG *[Ref. Harrison 19/e, p 2007, 18/e, p 2550]*

8. **Presence of HBeAg in patients with hepatitis indicates:** [AIIMS 92]
   a. Simple carriers b. Late convalescence
   c. High infectivity d. Carriers status
   [Ref. Harrison 19/e, p 2008, 18/e, p 2550]

9. **Anti-HBsAb indicates:** [AIIMS 92]
   a. Resistance to hepatits B
   b. Acute infections
   c. Good prognosis
   d. Hepatocellular carcinoma
   [Ref. Harrison 17/e, p 1943,19/e, p 2017]

10. **Most common route of spread of hepatitis E is:**
    a. Sex b. Feco-oral [AI 93]
    c. Blood transfusion d. IV injections
    [Ref. Ananthanarayan 8/e, p 546, 9/e, p 550]

11. **Hepatitis B is not transmitted by:** [AIIMS 93]
    a. Blood transfusion b. Pasteurized albumin
    c. Cryoprecipitate d. Sexual contact
    [Ref. Harrison 19/e, p 2006]

12. **Which of the following hepatitis has poor prognosis:**
    a. Hepatitis A b. Hepatitis B [UP 05]
    c. Non-A, Non-B type d. Hepatitis C
    [Ref. Harrison 19/e, p 2013, 18/e, p 2546]

13. **In chronic hepatitis B infection, which one of the following markers is indicative of active viral replication and the corresponding risk of disease transmission?**
    a. HBe antigen b. HBs antigen [UPSC 07]
    c. Anti-HBe d. Anti-HBs
    [Ref. Harrison 19/e, p 2008]

14. **Prophylactic vaccines are available for hepatitis:** [UP 08]
    a. Hepatitis A,B
    b. Hepatitis B,C
    c. Hepatitis C,D
    d. Hepatitis A, D *[Ref. Harrison 19/e, p 2012, 18/e, p 2546]*

Vaccine is available for hepatitis A, hepatitis B and hepatitis C

15. **HBV is associated with all of the following except:** [SGPGI 05]
    a. Hepatic cancer
    b. Chronic hepatitis
    c. Hepatic adenoma
    d. Cirrhosis *[Ref. Ananthanarayan 8/e, p 542, 9/e, p 550]*

Answers 1. a. Hepatitis C 2. a. Hepatitis C virus 3. a and b ... 4. c. Feco-oral ... 5. a. 0.5 ppm ...
6. d. Hepatitis E 7. b. HBsAg + ... 8. c. High infectivity 9. a. Resistance ... 10. b. Feco-oral
11. b. Pasteurized ... 12. b. Hepatitis B 13. a. HBe ... 14. a. Hepatitis ... 15. c. Hepatic ...

16. Route of transmission of hepatitis E virus is: [DNB 04]
a. Skin b. Faeco-oral
c. Blood d. Sexual contact
[Ref. Ananthanarayan 8/e, p 546, 9/e, p 550]

17. Hepatitis B virus vaccine contain: [NEET/DNB 09]
a. HBsAg b. HBc
c. HBe d. All of the above
[Ref. Ananthanarayan, 8/e, p 544, 9/e p 548]

18. Which of the following is not present in DANE particle?
a. Core antigen b. Surface antigen
c. p 53 d. None of above
[Ref. Ananthanarayan 8/e, p 539, 9/e, p 543]

Complete hepatitis virus is called as Dane particle

19. Antibody present during window period of hepatitis:
a. IgM anti-HBc b. IgM anti-HBe [Delhi 06]
c. IgG anti-HBc d. Anti-HBs
[Ref. Harrison 19/e, p 2017]

20. Which is DNA virus? [RJ 06]
a. Hepatitis A b. Hepatitis B
c. Hepatitis C d. Hepatitis D
[Ref. Ananthanarayan 8/e, p 539, 9/e, p 545]

21. Dependent hepatitis virus is: [DNB 2013]
a. Hep A **b. Hep D**
c. Hep B d. Hep C
[Ref. Ananthanarayan 9/e p 549]

22. Choose the false statement regarding hepatitis G virus: [Karnataka 2001]
a. Also called GB virus
b. Blood-borne RNA virus
c. Mostly infected with C virus
d. Responds to Lamivudine
[Ref. Ananthanarayan 9/e p 950]

Hepatitis G virus:
- RNA virus belonging to flavivirus family
- Mode of transmission is same as that of HCV, i.e. blood borne
- Also called as GB virus
- Prevalence is higher in patient infected with HIV and HCV

23. Which of the following does not go into chronic hepatitis stage: [TN 2001]
a. HBV b. HCV
c. HDV d. HEV
[Ref. Harrison 19/e, p 2013, 18/e, p 2546]

24. Hepatitis E clinically resembles: [Orissa 05]
a. Hepatitis A
b. Hepatitis B
c. Hepatitis C
d. Hepatitis D
[Ref. Ananthanarayan 8/e, p 546, 9/e, p 550]

25. HBV present in India is: [Manipal 06]
a. Adw b. Ayw
c. Adr d. Ayr
[Ref. Ananthanarayan 8/e, p 539, 9/e, p 544]

- HBsAg exhibit antigenic diversity. It contains two different antigenic components:
  a. Common group reactive antigen 'a'
  b. Two pair of type specific antigen d-y, w-r
- Thus, there can be four antigenic subtypes.
  1. ayw- Common in West Asia, Northen India, Western India.
  2. Adw- Common in Europe and Australia
  3. Adr: Found in South and East India
  4. Ayr: Rarest

26. All the statements are correct about hepatitis viruses, except: [J & K 05]
a. Maximum chance of chronic infection is HCV
b. Pregnant woman with HEV has 10-20% chance of mortality
c. Vaccine is available only against HBV
d. HBV and HCV has oncogenic potential
[Ref. Harrison 19/e, p 2013, 18/e, p 2546]

27. HBV is associated with all of the following except: [SGPGI 05]
a. Hepatic cancer b. Chronic hepatitis
c. Hepatic adenoma d. Cirrhosis
[Ref. Harrison 19/e p 2011, 18/e, p 2546]

28. Hepatitis D virus: [MAHE 07]
a. Transmitted by fecoral route
b. Is a DNA virus
c. Has independent existence
d. Resembles some plant viruses
[Ref. Ananthanarayan 8/e, p 545, 9/e, p 549]

29. Hepatitis C virus resembles which of the following virus groups: [COMED 07, PGI 99, JK 01]
a. Picornoviruses
b. Herpes viruses
c. Hepadna viruses
d. Flavi viruses
[Ref. Ananthanarayan 8/e, p 545, 9/e, p 549]

Answers **16.** b. Faeco-oral **17.** a. HBsAg **18.** c. p 53 **19.** a. IgM anti ... **20.** b. Hepatitis B
21. b. Hep D **22.** d. Responds ... **23.** d. HEV ... **24.** a. Hepatitis A **25.** b and c...
26. c. Vaccine ... **27.** c. Hepatic ... **28.** d. Resembles ... **29.** d. Flavi ...

30. Transmission of hepatitis A virus occurs: **[PGI 99]**
a. One week before and one week after onset of symptom
b. 2 weeks before onset of symptom
c. 2 weeks after onset of symptom
d. 1 week after onset of symptom

[Ref. Park, 22/e, p 191]

31. Acute infection with HBV is characterized by:
a. HBsAg **[AI 99, AIIMS 96]**
b. Anti-HBsAg
c. IgM anti-HBcAg and HBsAg
d. Anti-HBcAg *[Ref. Harrison 19/e, p 2017, 18/e, p 2550]*

32. HBV all true, except: **[PGI 98]**
a. It is a DNA virus
b. Spreads by blood transfusions
c. HBsAg marker of infectivity
d. Least chance of chronicity

[Ref. Harrison, 19/e, p 2006, 2013, 18/e, p 2539]

33. Which of the following is not matched correctly:

a. Hepatitis D	Defective virus	**[DNB 11]**
b. Hepatitis C	Parenteral transmission	
c. Hepatitis B	RNA virus	
d. Hepatitis E	Fecoral transmission	

[Ref. Ananthanarayan 8/e, p 538, 9/e p 543]

Answers 30. b and d. 31. c. IgM anti-HBcAg 32. c and d 33. c. Hepatitis B

NEET Pattern Questions

1. **True about HCV include all except:**
   a. Highest rate of chronicity among all hepatitis viruses
   b. Can be cultured
   c. Diagnosed by detection of HCV RNA
   d. Transmitted through transfusion of infected food
   [Ref. Ananthanarayan, 9/e, p 549]

   HCV virus can not be grown in culture, but has been cloned in E. coli.

2. **All of the following hepatitis virus can be transmitted through blood except:**
   a. Hepatitis B
   b. Hepatitis C
   c. Hepatitis D
   d. Hepatitis E *[Ref. Ananthanarayan, 9/e, p 550]*

3. **Commonest causes of acute hepatitis in India:**
   a. Hepatitis B
   b. Hepatitis B + D
   c. Hepatitis C
   d. Hepatitis A *[Ref. Park 22/e, p 191]*

4. **HBV and HDV false is:**
   a. Both can infect simultaneously
   b. HDV can cause more serious infection due to super infection
   c. HDV can not infect in absence of HBV
   d. DNA virus *[Ananthanarayan, 9/e, p 549]*

5. **Serological testing of a patient shows HBsAg, Ig Manti HBc and HBeAg positive. The patient has:**
   a. Chronic hepatitis B with low infectivity
   b. Acute hepatitis B with high infectivity
   c. Chronic hepatitis B with high infectivity
   d. Acute on chronic hepatitis
   [Ananthanarayan, 9/e, p 548]

6. **Hepatitis C virus is:**
   a. Togavirus
   b. Flavivirus
   c. Filovirus
   d. Retrovirus *[Ananthanarayan, 9/e, p 549]*

7. **Which is SsRNA unenveloped virus:**
   a. HBV
   b. HEV
   c. HCV
   d. None *[Ananthanarayan, 9/e, p 550]*

8. **HDV is**
   a. SS RNA virus
   b. SS DNA virus
   c. DS RNA virus
   d. DS DNA virus *[Ananthanarayan, 9/e, p 549]*

   HDV is a defective SSRNA virus

9. **First antiboy to appear in hepatitis:**
   a. IgM anti HBe
   b. IgM anti HBc
   c. IgG anti HBe
   d. IgM anti HBs *[Ananthanarayan, 9/e, p 546]*

10. **All cause viral hepatitis except:**
    a. Measles
    b. EBV
    c. Rhinovirus
    d. Reovirus *[Ananthanarayan, 9/e, p 541, 560]*

    Reovirus have not been proved to cause any human disease.

11. **Best test to diagnose prodrome of Hepatitis A:**
    a. HAV in blood
    b. IgG anti-HAV
    c. IgM anti-HAV
    d. HAV in stool *[Harrison, 19/e, p 2016]*

12. **Hepatitis E usually affects:**
    a. Children
    b. Adults
    c. Old age
    d. Toddlers *[Harrison, 19/e, p 2015]*

    In Asia most infections occur in young adults and are usually linked to genotype 1 and 2. In non-endemic areas genotype 3 and 4 are predominant and seen mainly in older males (>60 years).

13. **Which flavivirus causes hepatitis in human:**
    a. Hepatitis A
    b. Hepatitis B
    c. Hepatitis C
    d. Hepatitis D *[Jawetz, 27/e, p 496]*

14. **E antigen (HBeAg) of hepatitis B virus is a product of which gene:**
    a. S
    b. C
    c. P
    d. X *[Ananthanarayan, 9/e, p 544]*

Answers 1. **b.** Can be cultured 2. **d.** Hepatitis E 3. **d.** Hepatitis A 4. **d.** DNA virus 5. **b.** Acute hepatitis B
6. **b.** Flavivirus 7. **b.** HEV 8. **a.** SS RNA virus 9. **b.** IgM anti HBc 10. **d.** Reovirus
11. **c.** IgM anti-HAV 12. **b.** Adults 13. **c.** Hepatitis C 14. **b.** C

15. **HCV virus is:**
    a. Enveloped DNA
    b. Enveloped RNA
    c. Nonenveloped DNA
    d. Nonenveloped RNA

[Jawetz, 27/e, p 496]

16. **DNA polymerase of HBV is encoded by which of the following:**
    a. S gene
    b. C gene
    c. P gene
    d. X gene

[Ananthanarayan, 9/e, p 544]

17. **Super carrier of HBV shows following serum markers:**
    a. HBsAg
    b. HbsAg + HBV DNA
    c. HbsAG + HBeAg + HBV DNA
    d. Anti-BHsAg + HBV DNA

18. **Defective hepatitis virus is:**
    a. HAV
    b. HBV
    c. HCV
    d. HDV

[Ananthanarayan, 9/e, p 549]

19. **Which is not parenterally transmitted:**
    a. HAV
    b. HBV
    c. HCV
    d. HDV

[Ananthanarayan, 9/e, p 541]

20. **Infectivity of HBV is indicated by:**
    a. HBeAg
    b. HBsAg
    c. HBcAg
    d. Anti-Hbc

[Ananthanarayan, 9/e, p 547]

21. **Which of the following hepatitis virus is transmitted by fecal-oral route:**
    a. HAV
    b. HBV
    c. HCV
    d. HDV

[Ananthanarayan, 9/e, p 541]

22. **Hepatitis A virus is:**
    a. Flavivirus
    b. Calcivirus
    c. Enterovirus
    d. Defective virus

[Ananthanarayan, 9/e, p 541]

23. **Marker for acute viral hepatitis caused by HBV:**
    a. IgM anti-HBc Ag
    b. IgG anti-HBc Ag
    c. IgM anti-HBs Ag
    d. IgG anti-HBs Ag

[Harrison, 9/e, p 2018]

24. **Which of the following does not indicate Heapatitis B replication?**
    a. HBcAg
    b. HBeAg
    c. HBV DNA
    d. Viral copies

[Harrison, 9/e, p 2007]

25. **Councilman body is seen in:**
    a. Molluscum contagiosum
    b. Rabies
    c. Granuloma inguinale
    d. Viral hepatitis

[Harrison, 19/e, p 2012]

Answers 15. b. Enveloped RNA 16. c. P gene 17. c. HbsAG + HBeAg + HBV DNA 18. d. HDV
19. a. HAV 20. a. HBeAg 21. a. HAV 22. c. Enterovirus
23. a. IgM anti-HBc Ag 24. a. HBsAg 25. d. Viral hepatitis

CHAPTER

28

HIV and Other Retrovirus

- Discovered in 1983 by Luc Montagenier who called it as lymphadenopathy associated virus. The term HIV was given on 1986.
- Causative agent of **AIDS** = Slim disease.
- Belongs to *family* ***retroviridae;*** *subfamily* ***lentiviridae.***
- Two types are found HIV 1 and HIV 2. ***HIV 1 is most common cause*** of ***AIDS in world.***
- HIV 1 is more virulent than HIV 2.
- *Pan troglodyte troglodytes species of chimpanzees are natural reservoir of HIV 1.*

.... Harrison 17/e, p 1138, 19/e 1216

MORPHOLOGY AND REPLICATION CYCLE

- Spherical ***enveloped*** virus. Nucleocapsid has ***icosahedral*** structure.
- Virus contain *external spikes formed by the* ***two major envelope protein*** *— the external gp 120* and *transmembrane gp - 41.* gp 120 is the major envelope antigen.
- Genome is composed of two identical single stranded positive sense RNA copies.
- ***Main genes are:***
  - **gag** – Determines *the core and shell of virus.* Codes for precursor protein p55 which is cleaved into three proteins p15, p18 and p24.
  - **env** – Determines *the synthesis of envelope glycoprotein.* gp 160 which is then cleaved into gp 120 and gp 41.
  - **pol** – Codes for *polymerase reverse transcriptase* and other viral enzymes.

Note: Reverse transcriptase is RNA dependent DNA polymerase.
Inviral envelope lipid is derived from host cell membrane and glycoprotein are virus coded.

Other genes are: tat, rev, jej, vif, vpu, vpx, vpr, LTR.

- The major difference between genomes of **HIV 1** and **HIV 2** is HIV 2 lacks vpu gene and has vpx gene which is not present in HIV 1.

Antigenic variation and molecular heterogeneity

- ***HIV is highly mutable virus.***
- The variability of HIV is believed to be due to error prone nature of reverse transcription.

- There are three groups of HIV -1.
  - **Group M** ***(Most of infection), Group O and Group N.*** There are *nine subtypes* of M group:

    ..... Harrison 19/e, p 1216
    - **Subtype C is** *most prevelant* **worldwide.**
    - ***In India and China*** *also subtype* **C** *is most prevelant.*
    - In Europe, Australia and America subtype B is predominant.
- It is not uncommon to find recombinants form (CRF). CRF (circulating recombinant form) are generated by infection of a individual with two subtype which then remcombine and create a virus with selective advantage. *AE form is most prevalent CRF.*

MODES OF TRANSMISSION

A. Sexual transmission

- The *most common* **mode** of transmission worldwide is **heterosexual transmission.**
- Chance of infection from *male to female* is **twice** as from *female to male.*
- The overall risk of transmission ranges from 0.04-0.38% per coital act for female to male and 0.08 to 0.6 per act for male to female per act. Risk of HIV transmission via unprotected anal intercourse was estimated to be 1.4% for both men and women.

I

HIV:

- SS RNA virus belongs to retroviridae, subfamily lentiviridae.
- Two types HIV1 (more common) and HIV 2
- Main genes are:
  - gag (determine core and shell)
  - env (determine envelope)
  - pol (determine polymerase)
- HIV1: Three groups group M (most common) N and O
- There are 9 subtypes of M group, subtype C being most common.

Mnemonic: Learn as 1 MC

I

Major Antigens of HIV

Envelope antigen

Gp 120 : Spike antigen
Gp 41 : Transmembrane pedicle protein

Shell antigen

P18 : Nucleocapsid protein

Core antigen

P24 : Principal core antigen
P15, P55 : other core antigen

Polymerase antigen:

P31, P51, P66

I

Mode of Transmission:

- Sexual: MC, but risk of transmission is least.
- Blood and blood product: Carries (>95%) highest risk of transmission
- Vertical (risk is 30%).

I
Mode of transmission:
- Sexual → Blood/blood proe-ducts → Perinatal

B. Transmission by blood and blood products

- 2nd most common mode
- Transmission of whole blood. Packed red cells, platelets, leucocytes and plasma are all capable of transmitting HIV infection.
- Hyperimmunoglobulin, hepatitis B immunoglobulin, plasma derived heptatitis B vaccine and Rh immunoglobulin *have not been* associated with transmission of HIV infection.
- Risk of gettng HIV infection from **transfusion of a unit of infected blood is > 95%.**

C. Maternal fetal transmission: **Risk is 30%**

- Third most common mode
- Occurs ***most commonly*** in perinatal period.
- Cesarean section decrease risk of transmission.
- Risk of infection is high if the mother is newly infected or if she has already developed AIDS.
- ***Vitamin A deficiency*** increase risk of transmission.
- Exclusive breastfeeding carries lower risk of transmission than mixed feeding.

..... Harrison 18/e, p 1615

I
Pathogenesis:
- Quantitative and qualita-tive deficiency of helper or inducer T cell
- Co-receptor for HIV CCR-5 and CXCR4

- Presence of mastitis, low maternal CD4+ T cells counts and maternal vitamin A deficiency increase risk of transmission.

D. Transmission by other body fluids:

- HIV can be isolated in low titres from saliva, but *saliva can not transmit* HIV infection *due to presence* of endogenous antiviral factors of which most important ***is secretory leukocyte protease inhibitor (SLPI).***

PATHOGENESIS

- The hallmark of HIV disease is a profound immunodeficiency due to quantitative and qualtitative deficiency of helper or inducer T cells (CD4 - T cells).
- ***Replication begins*** with the high affinity ***binding of gp120 with CD-4*** (Present on CD4 + T cells and monocyte macrophage lineage cells). After binding with CD-4, gp120 undergoes conformational change that facilitates binding of corecepter. The major corecepter for HIV-1 are CCR-5 (receptor for chemokine RANTES, MIP1a and MIP1β) and CXCR-4 (the receptor for chemokine SDF-1)
- Strains of HIV that utilize ***CCR-5 as coreceptor,*** usually infect ***macrophages*** and are referred as R-5 virus.
- Strains of HIV that utilize ***CXC R4*** usually infects ***lymphocytes*** and are referred as X-4 virus.
- Many viral strains are dual tropic (utilize both CCR-5 and CXCR-4) which are referred as *R5X4 virus.*
- After fusion, HIV genome RNA is uncoated and internalized into target cell. The reverse transcriptase catalyze the reverse transcription of RNA to DNA. DNA so formed integrates with host cell chromosome through the action of virus encoded enzyme integrase.
- ***Primary HIV infection and Initial Viremia:***
  - Dendritic cells play an important role in the initiation of HIV infection due to presence of lectin called DC-SIGN which binds with high affinity to HIV envelope.
- ***Chronic and Persistant Infection:***
  - Establishment of chronic infection is due to the ability of virus to mutate.
  - Evolution of mutants that escape control by CD8 + cytolytic T lymphocytes is critical for progression of HIV infection.
  - Another mechanism is the down regulation of HLA class 1 molecules resulting in lack of ability of CD8 + CTL to kill the infected target cell.

I
Cellular targets of HIV:
- T cells CD_4, Monocyte-Mac-rophage, dendritic langer-hans cells Few B cells

- *Cellular target of HIV:*
  - **CD 4 + Helper = Inducer T cell** *(Primary target)*
  - Monocye - Macrophages (10-15%)
  - Dendritic, langerhans cells
  - Few B cells (5-10%).

CLINICAL FEATURES

- **Acute HIV infection** with in 3-6 weeks of infection.
  - Infectious mononucleosis like symptoms.
  - In most patient it is followed by prolonged period of clinical latency.
- **Asymptomatic stage** = Latent infection:
  - Median time of asymptomatic stage is ~ *10 years.*
  - Rate of *disease progression* can be directly correlated with *HIV RNA levels.*
  - During this stage rate of CD4 + T cell decline is ≈ 50 µl/yr.
  - ***Any HIV infected individual with CD4 + T cell count < 200/µl has AIDS by definition.***
- **Persistent generalized lymphadenopathy:** Presence of enlarged lymph nodes at least 1 cm in diameter in two or more non-coniguous extra-inguinal sites, that persist for atleast three months without any other attributable cause.
- **AIDS related complex: (ARC):** ARC includes patients with considerable immunodeficiency suffering from various constitutional symptoms or minor opportunistic infection.
- **Symptomatic Disease: AIDS**

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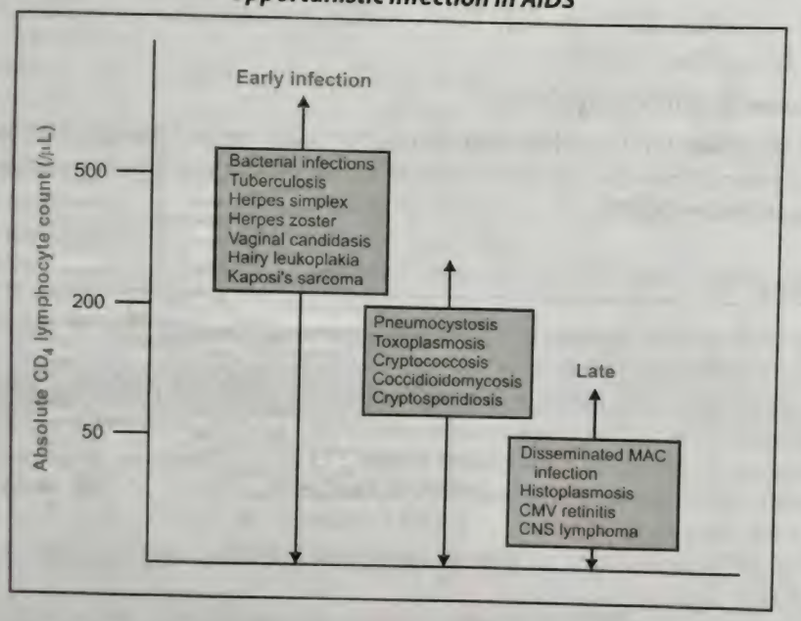
- Asymptomatic stage of HIV last for about 10 years
- Any HIV + individual with CD4 + T cell count < 200/µl has AIDS by definition

Opportunistic Infection in AIDS



AIDS

AIDS can lead to infection/disturbance in almost every organ system.

*A. **Disease of Respiratory System***

- Sinusitis
  - *Most commonly* maxillary sinus is involved. ***MC*** causative organism are ***H. influenzae*** and ***Streptococcus.***
- Pulmonary manifestation
  - ***MC*** pulmonary manifestation is pneumonia. The *most common* cause of pneumonia is ***P. carinii.***
  - Pneumocystis pneumonia present with fever, cough and shortness of breath but hypoxemia may be severe.
  - Chest X-ray shows diffuse or perihilar infiltrate (characteristic of *P. carnii*) but only in 2/3rd of patient. Chest X-ray might be normal in 10-15% of cases.
  - Large pleural effusion are uncommon and its presence suggest some other cause
  - Definitive diagnosis is made by direct fluoroscence antibody (DFA) test of induced sputum.
    - *MC bacterial cause of pneumonia in HIV patient are H. influenzae, streptococci.*
    - Tuberculosis-HIV increase risk of developing active TB by factor of 100. Can be seen even in high CD-4 count. Affect upper lobe (when CD-4 count is high), diffuse or lower lobe when CD-4 count is low. *...Harrison 19/e 1254*

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Clinical Manifestation:
- **Respiratory:** MC is pneumonia. MC cause P. carini.
- **Meningitis:** MC cause cryptococcus
- **Esophagitis:** MC cause Candida
- **Blindness:** MC cause CMV retinitis

- *In India, mycobacterium tuberculosis is MC opportunistic infection in AIDS patient.*
  - Atypical mycobacterial infection - *MC* are *M. avium* or *M. intracellulare* species (MAC) occur ***when CD-4 < 50/µl.***
  - *Mostly present as disseminated infection.*
- ***Fungal infection***- Coccidiodes immunitis; *Aspergillosis; Histoplasmosis.*
- *Idiopathic interstitial pneumonia*
- Lymphoid interstitial pneumonia (LIP) and nonspecific intestinal pneumonitis.

B. *Disease of CVS*

It includes:
- *HIV associated cardiomyopathy*—Dilated cardiomyopathy associated with CHF due to direct consequence of HIV infection.
- Pericardial effusion, cardiac tamponade
- Pericarditis, non-bacterial thrombolic endocarditis
- *Lipodystrophy syndrome*—Due to adverse effect of antiretroviral therapy.

C. *Disease of Oropharynx and GIT*

- *Oral lesions*
  - Thrush - Due to candida
  - Hairy leukoplakia - Due to EBV *(not a premalignant condition)*
  - Aphthous ulcer.
- *Esophagitis* Causative organism are:
  - Candida
  - CMV: Single large ulcer
  - HSV: Multiple small ulcer.
- ***Intestinal Infection***

  Causes of diarrhea in AIDS patient.
  - Bacterial : *Salmonella, Shigella, Campylobacter jejuni.*
  - Fungal : *Histoplasmosis, Coccidiodomycosis* and Pencillosis cause fever and diarrhea.
  - Protozoa : *Cryptosporidia, Microsporidia, Isospora belli*
  - Viral : CMV, HIV, Enteropathy

D. *Disease of genitourinary system*

- *Characterized by proteinuria*
- HIV induced nephropathy is a direct consequence of HIV infection
- Seen when CD-4 + T cell is below 20%, however can be seen in earlier stage too
- Diagnosis is made by biopsy which shows focal segmental glomerulosclerosis (80%) and mesangial proliferation.

E. *Disease of Hematopoietic system*

- *Anemia*—***MC*** hematologic abnormality.
- *Thrombocytopenia*—Due to platelet specific antibody or as a direct result of HIV on megakaryocytes.
- *Lymphadenopathy and Leukopenia.*

F. *Dermatologic disease*

- Dermatologic problems occur in >90% of patients with HIV infection.
- Folliculitis is the most prevalent dermatologic disorder in patients with HIV infection. Seen when CD4 + T cell counts < 200 cells/µl.
- Seborrheic dermatitis occurs in up to 50% of patient with HIV infection.
- Psoriasis and ichthyosis, though not increased in frequency, if occurs severity is more than normal population.
- HSV infection is the most common cause of genital lesion in AIDS patient.

G. *Neurologic Manifestations*

- ***MC*** opportunistic infection that involve CNS are *Toxoplasmosis; Cryptococcus.*
- *Meningitis*—***MC*** cause *Cryptococcus neoformans.*

- *HIV encephalopathy* = AIDS dementia complex. Subcortical dementia due *to direct effect of HIV.*
- *Seizures* – ***MC*** cause Toxoplasmosis.
- *Myelopathy* – Associated with CMV infection.
- *Peripheral neuropathy, multiple cranial nerve palsies*
- *Myopathy* – Due to direct effect of HIV or due to drugs.

H. Disease of endocrine system

- MC is lipodystropy secondary to ART
- Thyroid function alteration
- Hypogonadism

I. Ophthalmologic disease

- ***MC*** fundoscopic finding is cotton wool spot due to retinal ischemia.
- ***MC*** cause of **blindness** in HIV patient is CMV retinitis *(characteristic appearance – perivascular hemorrhage and exudate called as cottage and cheese appearance)* – occurs when CD-4 < 50/µl.
- Acute retinal necrosis syndrome = Progressive Outer retinal necrosis (PORN) - Due to HSV and varicella zoster.

J. Neoplastic disease

- *Kaposi's sarcoma* – Multicentric neoplasm of vascular origin appearing in skin, mucous membranes and viscera.
- *Non-Hodgkin's lymphoma:*
  - Immunoblastic lymphoma (***MC*** lymphoma in HIV patient)
  - Burkitt's lymphoma
  - Primary CNS – Usually associated with EBV.

- *AIDS increase incidence of:*

Hodgkin's disease	Multiple myeloma	Cervical Ca
Leukemia	Melanoma	Oral Ca
Brain tumor	Anal Ca	
Lung Ca	Testicular Ca	

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- HHV-8 has bee strongly implicated as a viral factor in the pathogenesis of KS

PEDIATRIC AIDS

- Recurrent bacterial infections are more common in children.
- Pneumonia caused by *pneumocystis* carinii is the ***MC*** **AIDS** defining diagnosis in childen with unrecognized *HIV infection.*
- Tumor *most commonly* associated are ***Non-Hodgkin's lymphoma.***

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- **Kaposi sarcoma**:Neoplasm of vascular origin
- Commonest lymphoma in AIDS patient is Immunoblastic lymphoma

DIAGNOSIS OF HIV INFECTION

Clinical

I. WHO case definition for AIDS surveillance:

Any individual (>12 years of age) is considered to have AIDS if at least 2 of the following major signs are present in combination with at least 1 minor sign.

Major signs:	• Weight loss ≥ 10% of body weight. • Chronic diarrhea for more than 1 months. • Prolonged fever for more than 1 month (intermittent or constant).
Minor signs:	• Persistent cough for more than 1 month. • Generalized pruritic dermatitis. • History of herpes zoster. • Oropharyngeal candidiasis. • Chronic progressive or disseminated herpes simplex infection. • Generalized lymphadenopathy.

The presence of either generalized Kaposi's sarcoma or cryptococcal meningitis is sufficient for the diagnosis of **AIDS** for surveillance purposes.

II. Expanded WHO case definition for AIDS surveillance:

For the purposes of **AIDS** surveillance an adult or adolescent (>12 years of age) is considered to have **AIDS** if a test for HIV antibody gives a positive result and one or more of the following conditions are present:

- ≥ 10% body weight loss or cachexia, with diarrhea or fever, or both, intermittent or constant, for at least 1 month, not known to be due to a condition unrelated to HIV infection.
- Cryptococcal meningitis.
- Pulmonary or extrapulmonary tuberculosis.
- Neurological impairment that is sufficient to prevent independent daily activities, not known to be due to a condition unrelated to HIV infection.
- Candidiasis of the esophagus.
- Clinically diagnosed life-threatening or recurrent episodes of pneumonia, with or without etiological confirmation.

Diagnosis:
- **Screening:** ELISA.
- **Further confirmation:** Western blot
- **RTPCR:** Sensitive for both diagnosis and monitoring

Monitoring:
- **CD+ cell count:** Best indicator of immediate status
- **HIV RNA:** Determine disease progression.

LABORATORY DIAGNOSIS

Laboratory diagnosis	
Demonstration of Antibody	**Demonstration of HIV or its components**
• ELISA **(sensitive and best screening test)**	• **Antigen detection** p-24 is earliest virus marker to appear in blood
• Western blot (specific) (demonstrate antibody to products of all major HIV gene)	• **Virus isolation**—By cultivation of patient lymphocyte with uninfected lymphocyte in present of IL-2
• Modern 4th generation EIA kit combines antibody detection with p24 antigen assay	• PCR—Gold standard for diagnosis in all stages of HIV – DNA PCR; RNA PCR, RT PCR – *RT PCR is most sensitive and best.*

- RT PCR is used as a diagnostic and prognostic tool and has become a technique of choice for studies of sequence diversity and microbial resistance to antiretroviral agents.
- **Nucleic acid amplification detection:** Reverse transcriptase PCR, branched DNA (b DNA) and nucleic acid sequence based amplification. (NSABA)
- **Among all RTPCR is most sensitive which can detect upto 40 copies/ml.**

Characteristics of Tests for Direct Detection of HIV		
Test	**Technique**	**Sensitivity**
Immune complex-dissociated p24 antigen capture assay	Measurement of levels of HIV-1 core protein in an EIA-based format following dissociation of antigen-antibody complexes by weak acid treatment	Positive in 50% of patients, detects down to 15 pg/mL of p 24 protein
HIV RNA by PCR	PCR amplification of cDNA generated from viral RNA (target amplification)	Reliable to 40 copies/mL of HIV RNA
HIV RNA by bDNA	Measurement of levels of particle-associated HIV RNA in nucleic acid capture assay employing signal amplification	Reliable to 50 copies/mL of HIV RNA
HIV RNA by NASBA	Isothermic nucleic acid amplification with internal controls	Reliable to 80 copies/mL of HIV RNA

Note: In patients in whom HIV infection is suspected, the appropriate initial test is the EIA. In case of strong suspecion and to make early diagnosis p24 antigen detection is recommended

Diagnosis of HIV Infection in Newborn

- The presence of anti-HIV antibody (ELISA) is not diagnostic of infection until after 18 month of age.
- Diagnosis during first few month of life is made by detection of HIV DNA with PCR (Best).
- Other methods are p-24 antigen detection and HIV culture.

Lab Monitoring of Patient with HIV Infection

- *CD4 + T cell count* – Best indication of immediate state of immunologic competence of patient with HIV infection.
- *HIV RNA determination* – By RT PCR and bDNA assay.
  Determine disease progression. Should be monitored every 3-4 months.
- *HIV resistance testing.*

TREATMENT

As per WHO recommendations 2010	
When to start	All adolescents and adults including pregnant women with HIV infection and CD4 counts of ≤350 cells/mm3, should start ART, regardless of the presence or absence of clinical symptoms. Those with severe or advanced clinical disease (WHO clinical stage 3 or 4) should start ART irrespective of their CD4 cell count.
What to be given	**Target Population** / **Preferred options** Adults and adolescents – AZT or TDF+ 3TC or FTC + EFV or NVP Pregnant women – AZT + 3TC + EFV or NVP HIV/TB coinfection – AZT or TDF + 3TC or FTC + EFV HIV/HBV coinfection – TDF + 3TC or FTC + EFV or NVP
Laboratory monitoring	All patients should have access to CD4 cell-count testing to optimize pre-ART care and ART management. HIVRNA (viral-load) testing is recommended to confirm suspected treatment failure. Drug toxicity monitoring should be symptom-directed.

Nucleoside reverse transcriptase inhibitor (NRTI)	Protease inhibitors	Non-nucleoside reverse transcriptase inhibitors (NNRT I)
AZT = zidovudine ddI = didanosine ddC = zalcitabine d4T = stavudine 3TC = lamivudine TDF = Tenofovir ABC = Abocavir	Ritonavir Indinavir (IDV) Nelfinavir Saquinavir (SQV) **Mnemonic: RIN shakti**	Delaviridine Nevirapine (NVP) Efavirenz (EFV)

WHO recommendations for initiating antiretroviral treatment in infants and children				
Criteria to start ART in infants and children				
Age	Infants	1-3 years	3-5 years	> 5 years
% CD4	All	< 20	< 20	< 20
Absolute CD4*		< 750 mm^3	< 350 mm^3	As in adults

* Absolute CD4 count is naturally less constant and more age-dependent than % CD4; it is not therefore appropriate to define a single threshold

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- Irrespective of CD4, ART should be given to all infants
- For post exposure prophylaxis Zidovudine, lamuvidine and nelfinavir should be administered for four weeks

Treatment of Opportunistic Infection		
Infection	**1st line treatment**	**Alternative treatment**
• Pneumocystic jeroveci	Trimethoprime + Sulphamethoxazole	Pentamidine
• Toxoplasmosis	Pyrimethamine + Sulphadiazine	Pyrimethamine + lindamycin
• Cryptococcus	Amphotericin + Flucytosine	Amphotericin
• Candida – Mucosal – Systemic	Clotrimazole/Flucytosine Amphotericin	Ketoconazole
• CMV	Gancyclovir	Fascarnet
• H simplex – Oral – Encephalitis	Acyclovir Acyclovir	Vidarabine
• Herpes zoster – Local – Disseminated	Acyclovir Acyclovir	– Vidarabine

POSTEXPOSURE PROPHYLACTIC TREATMENT

- Antiretroviral drugs started with hours following accidental exposure reduce the chances aquiring infection by 75%. Following regimen is recommended.
  - A combination of two nucleoside analogase reverse transcriptase inhibitor ***(mostly zidovudine + lamuvidine)*** for 4 weeks for routine exposure.
  - For high-risk exposure or if the source individual has advanced AIDS protease inhibitor nelfinavir should be added.
- Clinically all cases are considered high-risk and high-risk regime is given to all cases.
- If the source individual has failed on zidovudine + lamuvidine combination than stavudine + didinosine should be used instead of AZT + lamuvidine.

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- **HTLV 1:** Tropical spastic paraperesis and Adult T cell lymphoma
- **HTLV 2:** Virus searching for disease

OTHER RETROVIRUS

Human T cell lymphotrophic virus 1 (HTLV - 1):

- Also called as adult T cell lymphoma virus type I (ATLV 1)
- Causative agent of
  - Adult T cell lymphoma
  - Tropical spastic paraperisis.

Human T cells lymphotrophic virus II (HTLV - 2):

- Thought to be as a virus searching for disease.
- Associated with some cases of T cell varient of hairy cell leukemia.

Multiple Choice Questions

1. **HIV can be detected and confirmed by:** [AI 05]
   a. Polymerase chain reaction (PCR)
   b. Reverse transcriptase - PCR
   c. Real time PCR
   d. Mimic PCR

2. **An HIV patient complains fo visual disturbances. Fundal examination shows bilateral retinal exudates and perivascular hemorrhages. Which of the following viruses are most likely to be responsible for this retinitis:** [AI 04]
   a. Herpes simplex retinitis
   b. Human herpes virus
   c. Cytomegalovirus
   d. EBV

3. **Which one of following is true regarding HIV infection?** [AI 04]
   a. Following needle stick injury infectivity is reduced by administration of nucleoside analogues
   b. CD4 counts are the best predictors of disease progression
   c. Infected T cells survive for a month in infected patients
   d. In latent phase HIV has minimal replication

4. **HIV virus has:** [AI 02]
   a. Single stranded DNA
   b. Single stranded RNA
   c. Double stranded DNA
   d. Double stranded RNA

5. **Regarding HIV which of the following is not true?**
   a. It is a DNA retrovirus [AI 02]
   b. Contains Reverse transcriptase
   c. May infect host CD 4 cells other than T lymphoctyes
   d. Causes a reduction in host CD 4 cells at late stage of disease

6. **CMV retinitis in HIV occurs when the CD4 counts fall below:** [AI 02]
   a. 50 b. 100
   c. 200 d. 150

7. **Regarding HIV infection, not true is:** [AI 01]
   a. p24 is used for early diagnosis
   b. Lysis of infected CD4 cells is seen
   c. Dendritic cells do not support replication
   d. Macrophage is a reservoir for the virus

8. **Reverse transcriptase sequence in HIV is best described as:** [AI 00]
   a. RNA - DNA - RNA b. DNA - RNA
   c. DNA - RNA - DNA d. RNA - DNA

9. **Multifocal tumor of vascular origin in a patient of AIDS:**
   a. Kaposis sarcoma b. Astrocytoma [AI 00]
   c. Gastric carcinoma d. Primary CNS lymphoma

10. **A patient with HIV has diarrhea with AFB +ve organism in stool. The most likely organism is:**
    a. *Mycobacterium avium intracellulare* [AI 00]
    b. *Mycobacterium TB*
    c. *Mycobacterium leprae*
    d. *Mycoplasmas*

11. **A patient comes to hospital with a history of sore throat, diarrhea and sexual contact 2 weeks before the best investigation to rule out HIV is:** [AI 00]
    a. p24 antigen assay b. ELISA
    c. Western blot d. Lymph node biopsy

12. **A person with AIDS related complex is most likely suffering from:** [AI 12]
    a. Oppurtinistic infection
    b. Cancer related to AIDS
    c. Generalized lymphadenopathy
    d. Herpes zoster

13. **All the following are true about HIV infection except:**
    a. Caused by an enveloped RNA virus [AI 95]
    b. Rate of killing is directly proportional to T4 molecules on cell surface
    c. Increased release of acid labile interferon
    d. Decreased delayed hypersensitivity activity reaction

14. **In HIV window period indicates:** [AIIMS 07]
    a. Time period between infection and onset of symptoms
    b. Time period between infection and detection of antibodies against HIV
    c. Time between infection and treatment
    d. Time between treatment and death

15. **A known HIV positive patient is admitted in an isolation ward after an abdominal surgery following an accident. The resident doctor who changed his dressing the next day found it to be soaked in blood. Which of the following would be the right method of choice of discarding the dressing?** [AIIMS 05]
    a. Pour 1% hypochlorite on the dressing material and send it for incineration in a appropriate bag
    b. Pour 5% hypochlorite on the dressing material and send it for incineration in a appropriate bag
    c. Put the dressing material directly in an appropriate bag and send for incineration
    d. Pour 2% lysol on the dressing material and send it for incineration in a appropriate bag

16. **Tissue of origin of Kaposi's sarcoma is:** [AIIMS 05]
a. Lymphoid b. Vascular
c. Neural d. Muscular

17. **Which of the following lesion is associated with HIV infection:** [AIIMS 04]
a. Hairy leukoplakia b. Erythroplakia
c. Oral lichen planus d. Bullous pemphigoid

18. **All of the following methods are used for the diagnosis of HIV infection in a 2 month old child except:** [AIIMS 03]
a. DNA PCR b. Viral culture
c. HIV ELISA d. p24 antigen assay

19. **A resident doctor sustained a needle stick injury while sampling blood of patient who is HIV positive. A decision is taken to often him postexposure prophylaxis. Which one of the following would be the best recommendation:** [AIIMS 03]
a. Zidovudine + Lamivudine for 4 weeks
b. Zidovudine + Lamivudine + Nevirapine for 4 weeks
c. Zidovudine + Lamivudine + Indinavir for 4 weeks
d. Zidovudine + Stavudine + nevirapine for 4 weeks

20. **HIV infects most commonly:** [AIIMS 00]
a. CD4 + helper cells b. CD8 + cells
c. Macrophage d. Neutrophil

21. **Which of the following gene is present in HIV genome?** [PGI 06]
a. Gag b. Tat
c. p500 d. Kinase
e. P24

22. **Fungal infection associated with AIDS patient are:** [PGI 03]
a. *Pneumocystis carini* b. *Penicilliuea marneffei*
c. *Candida* d. *Cryptococcus*
e. *Cryptosporidium*

23. **HIV infection is associated with:** [PGI 02]
a. A glandular fever like illness
b. Generalized lymphadenopathy
c. Gonococcal septicemia
d. Sinus disease
e. Presenile dementia

24. **Which of the following is HIV gene?** [PGI 02]
a. gag b. tat
c. p2500 d. kinase
e. p24

25. **Which of the following is an AIDS defining criteria according to WHO?** [PGI 01]
a. Generalized lymphadenopathy
b. Fever, weight loss and fatigue
c. *Pneumocystis carinii pneumonia*
d. *Mycobacterium avium* infection
e. Persistent diarrhea

26. **In diagnosis of AIDS, criteria include the following except:** [PGI 01]
a. CD 4 < 200
b. CD 8 < 500
c. CD 4: CD 8 = 1
d. Presence of any of the opportunistic infections tuberculosis, *Pneumocystis carinii*, cytome-galovirus
e. Western blot is positive.

27. **Persistent diarrhea in AIDS is caused by A/E:** [PGI 01]
a. *Microsporidia* b. *Cryptosporidium parvum*
c. *Cryptococcus* d. *Isospora belli*
e. *Giardia lambia*

28. **Important features of AIDS are:** [PGI 01]
a. Follicular tonsillitis b. Lichen planus
c. Oral candidiasis d. Hairy leukoplakia

29. **HIV gene is/are:** [PGI 01]
a. Gp73 b. p24
c. Gp120 d. Gp5
e. None

30. **True about HIV:** [PGI 00]
a. Not transmitted through semen
b. More chances of transmission during cesarean section than normal labour
c. More infectious than hepatitis B
d. Male to female transmission > female to male

31. **In India, most common cause of TB in HIV:**
a. *Myco. tuberculosis* [PGI 00]
b. *Myco. avium intracellulare*
c. *M. bovis*
d. *M. scrofulaceum*

32. **Receptor for HIV?** [AI 09]
a. CD4 b. CD3
c. CD5 d. CD56

33. **CNS lesion in HIV is commonly caused by:** [PGI June 09]
a. *Cryptococcus* b. *Toxoplasma*
c. *Neurocysticercosis* d. *Mucormycosis*
e. Lyme disease

34. **Which is NOT AIDS defining illness?** [PGI June 09]
a. *Oropharynx candidiasis*
b. *CMV retinitis*
c. *Primary CNS lymphoma*
d. *Kaposi's sarcoma*
e. *Cryptococcosis*

35. **Most common genital lesion in HIV patient is:** [AI 2010]
a. *Chlamydia* b. *Herpes*
c. *Syphilis* d. *Candida*

36. **HIV virus was discovered in** [AIIMS May 2014]
a. 1976 b. 1983
c. 1996 d. 1988

Explanations and References with Illustrative Answers

1. **Ans. (b) Reverse transcriptase PCR** *Ref. Harrison 19/e, p 1247, 18/e, p 1539*

Laboratory diagnosis of AIDS

a. Virus Isolation
- HIV can be cultured from lymphocytes of peripheral blood. The number of circulating infected cells vary with the stage of disease. High titres are found in advanced stage. The most sensitive virus isolation technique is to co-cultivate the test sample with uninfected nitrogen stimulated blood mononuclear cells. Viral growth is confirmed by testing culture supernatant fluid (after 7-14 days) for viral reverse trranscriptase activity or for virus specific antigens.
- Due to the time it requires, virus isolation is seldom used for diagnosis of AIDS. It is mainly indicated for lab characterization of virus and to determine clinical stage; as plasma viremia is a better correlate of clinical stage than the presence of any antibody.
- For determining clinical stage too, *viral nucleic acid* load estimation is rapid and has replaced virus culture.

b. Serology
- *Enzyme linked immunoassay* has a sensitivity and specificity above 98%. It is cheap and yield rapid result; these properties makes ELISA screening test of choice.
- When EIA tests are used for screening population with low prevelance of HIV infection, a positive test must be confirmed by a repeat test. If the repeat test is reactive, a confirmation test is performed to rule due false positive EIA result.
- Most commercial EIA Kit contains antigen from both HIV-1 and HIV-2
- Conditions associated with false positive EIA include antibodies to class II antigens (may be seen during pregnancy, post-transplantation), auto antibodies, hepatic disease, recent influenza vaccination, and acute viral infection.
- *Most widely used confirmation assay* is ***western blot*** in which antibody to specific viral proteins can be detected. Antibodies to viral core protein p24 or envelope glycoproteins gp41, gp120 or gp160 are most commonly detected.
- The mean time for seroconversion after HIV inection is 3-4 weeks, virtually all HIV infected individuals have detectable antibodies within 6 months. Means both ELISA and westernblot yied false negative result during the ***window period*** (*an interval required for seroconversion after HIV infections*) which usually range from 3 weeks - 6 months. Antiretroviral therapy during window period also delays sereconversion. X-linked agammaglobinemia is another condition in which antibody test may yield false negative result .

c. Detection of viral nucleic acid or Antigens
- RT PCR, DNAPCR and bDNA assays are commonly used to estimate viral RNA in clinical specimen.
- RT-PCR uses an enzymatic method to amplify HIV RNA
- bDNA assays amplifies viral RNA by sequential oligonucleotide hybridization steps

These molecular test are very sensitive and can be used for both diagnosis and plasma viral load estimation i.e. prognosis.
- Low level of circulating HIV-1 p24 antigen can be detected in plasma soon after infection. The antigen often become undetectable after formation of antibodies (because p-24 antigen is complexed with p24 antibody), but may reappear late in the course of infection. *This reappearance indicates a poor prognosis.*

2. **Ans. (c) Cytomegalovirus** *Ref. Harrison 19/e, p 1267, 18/e, p 1562*

Ophthalmologic manifestations of HIV infection
- The *MC* abnormal finding on fundus examination are cotton wool spots. They represent area of retinal ischemia secondary to microvascular disease. These lesions are not associated with visual loss.
- **CMV Retinitis:**
  - ***MC cause of vision loss in*** AIDS ***patient.***
  - Usually occurs when CD-4 <50/µl.
  - Usually present as painless progressive loss of vision, patient may also complain of floaters and scintillations.
  - The *characteristic* appearance is that *of perivascular hemorrhage and exudate* called as cottage and cheese appearance or pizza pie retinopathy

- It may be complicated by rheumatogenous retinal detachment.
- *Treatment:* Oral valganciclovir, IV ganciclovir—*DOC*

- **Acute retinal necrosis syndrome = Progressive outer retinal necrosis (PORN):**
  - Caused by herpes simplex virus, varicella zoster virus.
  - Associated with pain, keratopathy iritis.
  - Ophthalmologic examination show widespread pale grey lesions.
  - *DOC* is intravenous acyclovir.
- **Other secondary infections include:**
  - *P. carinii* — Cause lesion of choroid.
  - Toxoplasmosis — Cause chorioretinitis.

3. Ans. (a) Following needle stick injury infectivity is reduced by administration of nucleoside analogues

Ref. Harrison 19/e, p 1247, 18/e, p 1583; Park 22/e, p 327

"Four week treatment with AZT monotherapy after needle stick exposure to HIV among health care worker decreases the chance of their becoming infected by 79%."

Guideliness for postexposure prophylaxis

I. A combination of two nucleoside analogue reverse transcriptase inhibitors for 4 weeks for less severe exposures.
II. A combination of two nucleoside analogue RT inhibitor plus a protease inhibitor given for 4 weeks for more severe exposure.

Most clinical administer 2nd reginen in all cases

Factor associated with increased risk of occupational transmission

- Deep injury.
- Presence of visible blood on the instrument causing the exposure.
- Injury with the device that has been placed in vein or artery of source patient.
- Terminal illness of source patient.
- Lack of postexposure antiretroviral therapy in exposed health care worker.
- TB is another infection common to HIV infected population that can be transmitted to health care workers following needle stick. For this reason all healthcare workers should check there PPD status and receive 6 months of isoniozid treatment if their skin test is positive.

Other Options

Option 'b'

- *Best predictor to disease progression is HIV RNA estimation (not CD 4 + count.)* ..... *Harrison 19/e, p 1248*
- CD 4 + count are the *best* indicator of immediate state of immunologic competence.

Option 'd'

- **Clinical latency versus microbial latency**
  - Clinical latency should not be confused with microbial latency since virus replication is present in most patient.

So, ***option 'd'*** is wrong.

Remember: Test for monitoring of patient with HIV infection:
- CD 4 + T cell count
- HIV RNA determination—Best by RT-PCR
- HIV resistance testing.

4. Ans. (b) Single stranded RNA *Ref. Ananthanarayan 8/e, p 569, 9/e, p 571*

Classification of HIV (= HTLV III)

Family	–	*Retroviridae*
Subfamily	–	*Lenti virus*
Genome	–	*ss RNA positive sense.*

- Viron contain ***lipoprotein envelope*** and nucleocapsid is icosahedral in shape.
- ***Characteristic feature of retrovirus*** is presence of reverse transcriptase enzyme.
  - Viral RNA is transcribed by this enzyme first in to ss DNA then to dsDNA which gets integrated with host cell.
  - Thus in contrast to central dogma flow of information is RNA → DNA → RNA

5. **Ans. (a) It is DNA retrovirus** *Ref. Ananthanarayan 8/e, p 569, 9/e, p 571*

HIV is RNA retrovirus *(not DNA virus)*

- It primarily infect CD 4 + T cells, but can also infect other cells also which bear CD4 receptor on their surface. These include circulating dendritic cells; epidermal langerhans' cells; monocytes.

Retroviriae	
• Oncoviridae (oncogenic viruses)	– RNA tumor virus group (HTLV 1, HTLV 2 Rous sarcoma virus).
• Lentiviridae	– HIV 1, HIV 2 – Visna virus – Feline immunodeficiency virus
• Spumavirinae foamy virus	– Simian foamy virus – Human foamy virus

6. **Ans. (a) 50** *Ref. Harrison 19/e, p 1267, 18/e, p 1562*

Already explained

7. **Ans. (c) Dendritic cells do not support replication** *Ref. Ananthanarayan 9/e, p 574; Harrison 19/e, p 1230, 18/e, p 1529*

- **Cellular target of HIV**
  - CD 4+ lymphocyte and CD 4 + cells of monocyte and macrophage lineage are principal target of HIV.
  - Circulating dendritic cells – play important role in initiation of HIV infection.
  - Epidermal langerhans' cells.
  - 5 - 10% of B lymphocyte. ..... *Ananthanarayan, 9/e, p 574*
- Degree of cytopathicity of HIV for cells of the monocyte lineage *is low,* and HIV can replicate extensively in cells of monocyte lineage. Hence play a role in dissemination of HIV in the body and can serve as reservoir of HIV infection. ..... *Harrison, 19/e, p 1238*
- **p24 is the earliest virus marker** to appear in blood and is the one tested for.

8. **Ans. (a) RNA - DNA - RNA** *Ref. Ananthanarayan 9/e, p 571*

Already explained

9. **Ans. (a) Kaposi's sarcoma** *Ref. Harrison 19/e, p 1268, 1186, 18/e, p 1564*

Kaposi's sarcoma is a multicentric neoplasm of vascular origin consisting of multiple vascular nodules appearing in skin, mucous membrane and viscera.

Feature of Kaposi's sarcoma:

- Can develop at any stage of HIV infection, even in presence of normal CD-4 count.
- It is a manifestation *of* excess proliferation of spindle cells that are believed to be vascular origin. ..... *Harrison 18/e, p 1564*
- Development of KS is associated with Human herpes virus 8 or HHV - 8 is etiologic agent of KS.
- Clinically KS often appear in sun exposed areas, particularly tip of nose. The initial lesion is a small raised reddish purple nodule on skin *(MC* appear as raised nodules).
- LN involvement does not signify poor prognosis.

Treatment

- *Observation and optimization of antiretroviral therapy.*
- *Single or limited number of lesions*
  - Radiation
  - Intralesional vinblastine and Cryotherapy.
- *Extensive disease* Initial therapy:
  - Interferon - α (if CD 4 + T cells > 150/μl)
  - Liposomal daunorubicin.
  - Subsequent therapy:
    - Liposomal doxorubicin
    - Paclitaxel and Radiation treatment.
  - Combination chemotherapy
  - Radiation treatment

10. **Ans. (a)** ***Mycobacterium avium intracellulare*** *Ref. Harrison 19/e, p 1254, 18/e, p 1548*

MAC infection in AIDS patients

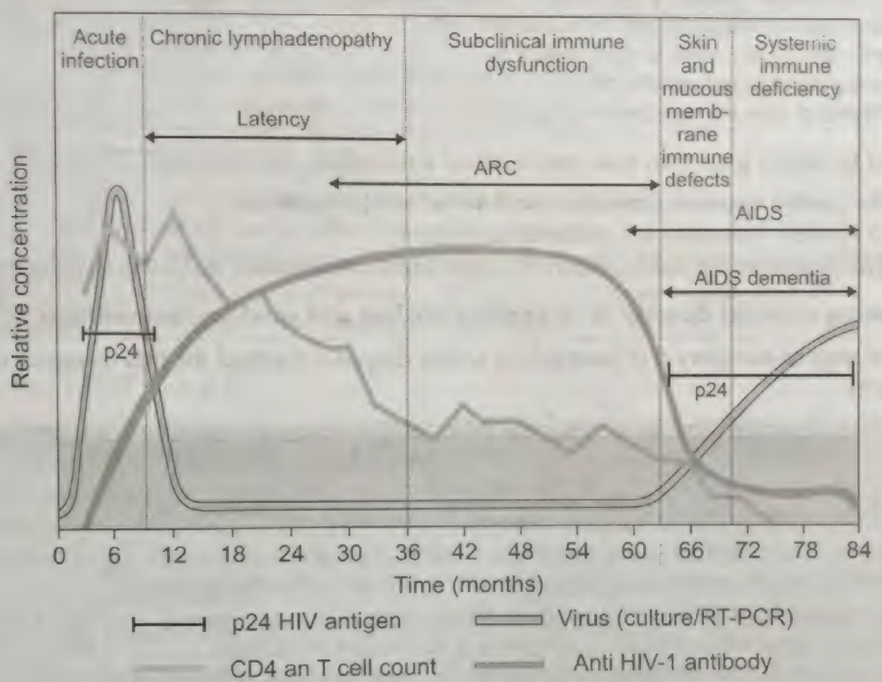
- MAC infection is the late complication of HIV infection occur when CD 4< 50/μl.

- MC presentation is disseminated disease with fever weight loss and night sweats. Other clinical features are:
  - Abdominal pain
  - Diarrhea
  - Lymphadenopathy.
- Diagnosis is made by demonstration of long, slender AFB in sputum, stool, blood or bone marrow.
- **Treatment:** *Clarithromycin + Ethambutol is treatment of choice.*

11. Ans. (a) p24 antigen assay *Ref. Ananthanarayan 8/e, p 577, 9/e, p 576*

The major core antigen p24 is the earliest virus marker to appear in blood and is the one tested for.

- p24 antigen assay is the most useful screening test for acute HIV syndrome as p24 antigen assay can detect those in windows period also.
- After appearance of IgM, p24 antigen disappears and remain undetectable, P24 antigen reappears when severe disease set in.



- Sequence of appearance of p24 antigen and antibodies after a massive HIV infection with time course of immune response, viremia, and disease resulting from HIV 1 infection.

12. Ans. (a) Opportunistic infection *Ref. Ananthanarayan 8/e, p 574, 9/e, p 575*

Natural course of HIV infection can be divided into following stages:

a. **Acute HIV infection:** Seen in 30% of individuals within 3-6 weeks of infection. They present with low grade fever, headache, malaise some time with rash and arthropathy resembling glandular fever. Spontaneous rresolution occurs within weeks. Tests for HIV antibodies are usually negative at the onset of illness but become positive during its course. So this stage is also called as seroconversion illness.

b. **Asymptomatic or Latent Infection:** Seen in all patients. This phase which lasts upto several years, patient remain asymptomatic. HIV antibody test is positive in this phase and patients are infectious. This period of clinical latency does not mean viral latency as virus multiplication goes on throughout.

c. **Persistant generalized lymphadenopathy:** This stage is defined as presence of enlarged lymphnodes at least 1 cm in diameter in two or more non-contagious extrainguinal sites, that persist for at least three months, in absence of any current illness or medication that may cause lymphadenopathy.

d. **AIDS related complex:** This group include patients with considerable immunodeficiency suffering from various constitutional symptoms or minor opportinistic infections like oral candidiasis herps zoster, hairy cell leukoplakia.

e. **AIDS:** This is the endstage representing the irreversible breakdown of immunodefence mechanisms leading to progressive opportunistic infection and malignancies.

Remember:
- During the period of clinical latency 10 billion HIV particles are produced and destroyed every day.
- Half life of virus in plasma is about 6 hours.
- Virus life cycle *(from the time of infecion of a cell to the production of new progeny that infect the next cell)* averages 2 6 days

13. **Ans. (c) Increased release of acid labile interferon** *Ref. Ananthanarayan 8/e, p 573, 9/e, p 574-575*

Infected T-4 cells do not appear to release normal amount of IL-2, IFNγ and other lymphokines.

γ Interferon is acid stable in contrast to other interferons.

Immunological abnormalities in HIV infection
I. Features that characterize AIDS • Lymphopenia • Selective T cell deficiency — Reduction in number of T4 (CD4) cells, Inversion of T4: T8 ratio. • Decreased delayed hypersensitivity on skin testing • Hypergammaglobulinemia — predominantly IgG and IgA; and IgM also in children. • Polyclonal activation of B cells and increased spontaneous secretion of Ig.
II. Other consistently observed features • Decreased in vitro lymphocyte proliferative response to mitogens and antigens. • Decreased cytotoxic response by T cells and NK cells • Decreased antibody response to new antigens. • Altered monocyte/macrophage function. • Elevated levels of immune complexes in serum.

14. **Ans. (b) Time period between infection and detection of antibodies** *Ref. Harrison 17/e, p 1164; Ananthanarayan 8/e, p 579*

Window period is the period between infection and detection (diagnosis).

- This range from 3 weeks - 2 months for antibody detection
- Detection by p24 antigen has decreased this to 16 days and subsequently to 12 days with nucleic acid testing.

15. **Ans. (c) Put the dressing material directly in an appropriate bag and send for incineration** *Ref. Park 22/e, p 738*

This type of waste belongs to category 6 of biomedical waste disposal method for this category is direct incineration without chemical treatment.

Categories of Biomedical Waste in India		
Option	**Waste category**	**Treatment and disposal**
Category No.1	Human Anatomical Waste (Human tissues, organs body parts)	Incineration / deep burial[2]
Category No. 2	Animal Waste (Animal tissue, organs, body parts blood and experimental animals used in research, waste generated by veterinary hospitals colleges.	Incineration / deep burial[2]
Category No. 3	Microbiology and Biotechnology Waste (Waste from laboratory cultures, stock or specimens of micro-organisms, live or attenuated vaccines, human and animal cell culture used in research and infectious agents from research and industrial laboratories, waste from production of biologicals, toxins, dishes and devices and for transfer of cultures)	Local autoclaving / microwaving / incineration
Category No. 4	Waste sharps (needle, syringes,scar pels, blades, glass, etc. that may cause puncture and cuts. This includes both used and unused sharps)	Disinefction (chemical treatment ≅ autoclaving/microwaving and mutation/shredding)
Category No. 5	Discarded medicines and cytotoxic drugs (wastes comprising of outdated, contaminated and discarded medicines)	Incineration ≅ destruction and drugs disposal in secured landfills
Category No. 6	Solid waste (items contaminated with blood, and fluids including cotton, dressings, soiled plaster casts, linen, beddings, other material contaminated with blood)	Incineration ≅ autoclaving/ microwaving
Category No. 7	Solid waste (wastes generated from disposable items other than the waste sharps such as tubings, catheters, intravenous sets, etc.)	Disinfection by chemical treatment ≅≅ autoclaving /microwaving and mutilation/shredding ##
Category No. 8	Liquid waste (waste generated from laboratory and washing, cleaning, housekeeping and disinfecting activities)	Disinfection by chemical treatment ≅≅ and discharge into drains
Category No. 9	Incineration ash (ash from incineration of any biomedical waste)	Disposal in municipal landfill
Category No. 10	Chemicals used in production of biologicals, chemicals used in disinfection, as insecticitdes, etc.	Chemical treatment ≅≅ and discharge into drains for liquids and secured landfill for solids.

≅≅ Chemical treatment using at least 1% hypochlorine solution or any other equipment chemical reagent. It must be ensured that chemical treatment ensures disinfection.

Multilation/shredding must be such so as to prevent unauthorized reuse.

≅ There will be no chemical pretreatment before incineration. Chlorinated plastics shall not be incinerated.

2 Deep burial shall be an option available only in towns with population less than lakhs and in rural areas.

Color coding and type of container for disposal of biomedical wastes		
Color coding	Type of container	Waste category
Yellow	Plastic bag	Cat. 1, Cat. 2, and Cat. 3, Cat. 6
Red	Disinfected container/plastic bag	Cat. 3, Cat. 6, Cat. 7
Blue/White translucent	Plastic bag/puncture proof container	Cat. 4, Cat. 7
Black	Plastic bag	Cat. 5, Cat. 9 and Cat. 10 (solid)

16. **Ans. (b) Vascular** *Ref. Harrison 19/e, p 1268, 18/e, p 1564*
Already explained

17. **Ans. (a) Hairy leukoplakia** *Ref. Harrison 19/e, p 1255, 18/e, p 1549*

Oral lesions in AIDS Patient	
1. Hairy leukoplakia	– Caused by EBV – White frond like lesion along lateral border of tongue – **Not a premalignant condition** – Treatment : Topical podophylin or systemic acyclovir.
2. Thrush	– Caused by Candida – White chessy exudate on erythematous mucosa in post oropharynx – **Most commonly** seen on soft palate – Diagnosed by direct examination of scraping for pseudohyphal elements.
3. Aphthous ulcer	– Painful ulcer of unknown etiology over posterior oropharynx – Thalidomide is an effective treatment.

18. **Ans. (c) HIV ELISA** *Ref. CPDT 16/e, p 55*
HIV ELISA is not useful for diagnosing HIV infection in newborn because IgG antibody of mother which has been transferred to neonate gives false positive result.

Diagnosis of HIV in newborn:	1. Detection of HIV DNA or RNA by PCR (Most effective)
	2. HIV culture
	3. HIV p24 antigen assay.

19. **Ans. (c) Zidovudine + Lamivudine + Indinavir for 4 weeks** *Ref. Harrison 19/e, p 1284, 18/e, p 1582*
Postexposure prophylaxis = Two nucleoside inhibitor + Protease inhibitor
So, the treatment is ***Zidovudine + Lamuvidine + Indinavir***
For details refer Ans. no. 3

20. **Ans. (a) CD4 + helper cells** *Ref. Harrison 18/e, p 1528, 19/e p 1236; Ananthanarayan 9/e, p 574*
Already explained

21. **Ans. (a) and (b) Gag and Tat** *Ref. Ananthanarayan 8/e, p 570, 9/e, p 571*

Genes of HIV

Genes coding for structural protein

- *gag gene* — determine the core and shell of virus
- *pol gene* — codes for reverse transcriptase and other enzymes endonuclease
- *env gene* — encodes the envelope glycoprotein

Regulatory gene

- *tat gene* – enhance expression of all viral gene
- *nef gene* – down regulating viral replication
- *rev gene* – enhancing expression of structural protein
- *vif gene* – influence infectivity of viral particle
- *vpu gene* – (present only in HIV - 1) ┐ Enhance maturation and release of progeny
- *vpx gene* – (present only in HIV - 2) ┘
- *vpr gene* – stimulate promoter region of virus
- *LTR sequence* – Giving promoter, enhancer, integration signal

Note: Detection of type specific sequences vpu and vpx is useful in distinguishing between HIV-1 and HIV-2

22. **Ans. (a, b, c) and (d) *Pneumocystis carinii; Penicillium marneffei; Candida* and *Cryptococcus*** *Ref. Harrison 19/e, p 1250-1255, 18/e, p 1547, 1551*

Fungal infection in AIDS patient are:

- ***Pneumocytis carinii (MC opportunistic infection in HIV patient)***
- **Cryptococcus neoformans *(MC cause of meningitis in HIV patient)***
- *Histoplasma*
- *Penicillium marneffei*
- *Coccidiodes immitis*
- *Aspergillosis* (pseudomembraneous bracheobronchitis in AIDS patient).
- Sporothrix.

Note: Cryptosporidium is a parasite not a fungus.

23. **Ans. (a, b, c) and (e) A glandular fever like illness; Generalized lymphadenopathy; Gonococcal septicemia; and Presenile dementia**

See below

Gonococcal septicemia is seen in terminal complement deficiency not in HIV patient.

I do not think that I should explain whole clinical feature here again. Just revise them from theory portion.

24. **Ans. (a) and (b) Gag and Tat** *Ref. Harrison 19/e, p 1219, 18/e, p 1508*

Already explained

25. **Ans. (a, b, c, d) and (e) All are correct options** *Ref. Park 22/e, p 322-323*

Guys, please see AIDS defining criteria from theory portion.

26. **Ans. (b) and (c) CD 8 < 500; and CD 4: CD 8 = 1** *Ref. Harrison 18/e, p 1543, 19/e, p 1250*

Any HIV infected individual (>6 years) with a CD-4 + T cell count of < 200/μl has AIDS by definition regardless of symptom or opportunistic infection.

27. **Ans. (c) and (e) *Cryptococcus*; and *Giardia lambia*** *Ref. Harrison 19/e, p 1257, 18/e, p 1550-1551*

Cause of diarrhea in HIV patient		
Bacterial	**Fungal**	**Parasitic**
– Shigella	– Histoplasmosis	– Cryptosporidia
– Salmonella	– Penicillosis	– Microsporidia
– Campylobacter	– Coccidioidomycosis	– Isospora belli

28. **Ans. (c) and (d) Oral candidiasis; and Hairy leukoplakia** *Ref. Harrison 19/e, p 1255, 18/e, p 1549*

Already explained

29. **Ans. (e) None** *Ref. Harrison 17/e, p 1140; Ananthanarayan 8/e, p 570, 9/e, p 571-572*

The options given are not gene but their protein products.

30. **Ans. (d) Male to female transmission > female to male** *Ref. Harrison 19/e, p 1220, 18/e, p 1510-1513*

Modes of transmission of AIDS

- ***Sexual Transmission***
  - *MC* mode of HIV transmission is heterosexual transmission.
  - Chances of HIV infection from male to female is twice
  - Anal intercourse carries higher risk of transmission.
  - Presence of STD increase risk of transmission.
  - Adolescent girl and women above 45 are more prone to get HIV infection.
- ***Transmission by blood and blood products***
  - Transfusion of whole blood, packed RBC, platelets, leukocytes and plasma are all capable of transmitting HIV infection.
  - Hyperimmune gamma globulin, hepatitis B immunoglobulin, plasma derived hepatitis B Vaccine and Rh immunoglobulin have not been associated with transmission of HIV infection.
- ***Maternal fetal transmission***
  - Risk of transmission is 30%.

- *Most common at the time of delivery.*
- *Cesarian section* decrease risk of transmission.
- Risk is more if mother has advanced AIDS or Acute HIV syndrome.
- In case of twin delivery, first born twin has more risk of getting infection than second born.
- *Breastfeeding can transmit infection,* but exclusive breastfeeding caries lower risk than mixed feeding.
- Presence of mastitis, Vitamin A deficiency increase risk of transmission via breastfeeding.

31. Ans. (a) *Mycobacterium tuberculosis* *Ref. Harrison 19/e, p 1254, 18/e, p 1548*

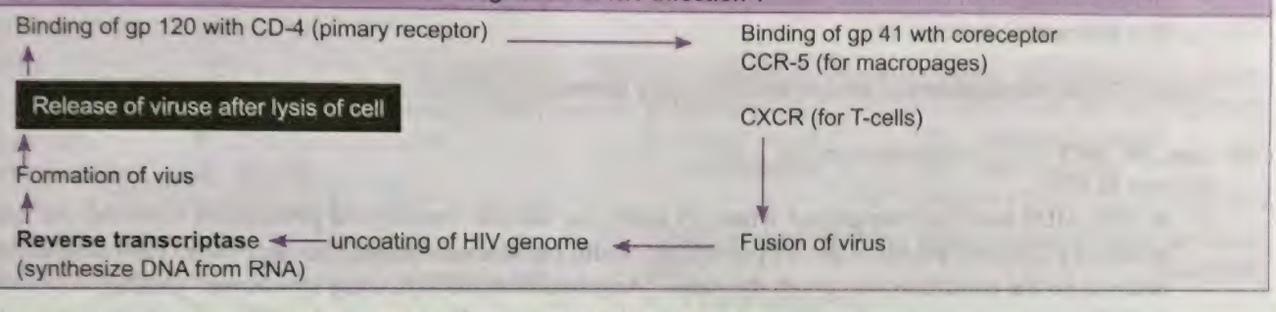
- HIV infection increase risk of developing active TB by a factor of 100.
- In developing countries where *M. tuberculosis* infection is most frequent, HIV infected individual have primary and secondary infection with the usual way.
- Opportunistic infection with *M. avium intracellulare* is *MC* opportunistic infection in west.

***So, it is clear that in developing countries like India MC mycobacterial infection is* M. tuberculosis.**

32. Ans. (a) CD4 *Ref. Ananthanarayan 8/e, p 572, 9/e, p 574*

The specific receptor of HIV virus is CD-4 antigen

Pathogenesis of HIV Infection 1



33. Ans. (a,b) *Cryptococcus, Toxoplasma* *Ref. Harrison 19/e, p 1263, 18/e, p 1558*

Neurologic disease in patients with HIV infection

Opportunistic infections
- Toxoplasmosis
- Cryptococcosis
- Progressive multifocal leukoencephalopathy
- Cytomegalovirus
- Syphilis
- Mycobacterium tuberculosis
- HTLV 1 infection

Neoplasms
- Primary CNS lymphoma
- Kaposi's sarcoma

Result of HIV-1 infection
- Aseptic meningitis
- HIV-associated neurocognitive impairment, including HIV encephalopathy/AIDS dementia complex

Myelopathy
- Vacuolar myelopathy
- Pure sensory ataxia
- Paresthesia/dysesthesia

Peripheral neuropathy
- Acute inflammatory demyelinating polyneuropathy (Guillain-Barré syndrome)
- Chronic inflammatory demyelinating polyneuropathy (CIDP) Monoeuritis multiplex
- Distal symmetric polyneuropathy

Myopathy

Remember:
- MC cause of meningitis in AIDS patient: Cryptococcus
  - Seizures in AIDS patient can be due to HIV encephalopathy (MC), cerebral toxoplasmosis, cryptococcal meningitis (8%) CNS lymphoma (15-30%), progressive multifocal leukoencephalopathy.

34. Ans. (a) Orpharynx candidiasis *Ref. Harrison 17/e, p 1138, Park 22/e, p 322*

Candidiasis of bronchi, trachea, lung, oesophagus comes under AIDS defining criteria.

Infections listed in the AIDS surveillance case definition
- Candidiasis of bronchi, trachea, lungs, oesophagus
- Coccidioidomycosis, disseminated or extrapulmonary

- Cryptococcosis, extrapulmonary
- Cryptosporidiosis, chronic, intestinal (> 1 month's duration)
- Cytomegalovirus disease (other than liver, spleen, or nodes)
- Cytomegalovirus retinitis (with loss of vision)
- Encephalopathy, HIV-related
- Herpes simplex: chronic ulcer(s) (> 1 month's duration); orbronchitis, pneumonia, or esophagitis
- Disseminated or extrapulmonary histoplasmosis
- Isosporiasts, chronic intestinal (> 1 month's duration)
- Mycobacterium avium complex or M.kansosil, disseminated or extrapulmonary
- Mycobacterium tuberculosis, any site (pulmonarya or extrapulmonary)
- Mycobacterium, other species or unidentitled species, disseminated or extrapulmonary.
- Pneumonocystis jiroved pneumonia
- Salmonella septicemia, recurrent
- Toxoplasmosis of brain
- Wasting syndrome due to HIV

35. Ans. (b) Herpes

See below

- Herpes simplex (in developed world) and chancroid (in Africa) are the most common cause of genital ulceration in HIV infected patients.

Note: Clinical manifestations of genital herpes are more severe and persistant in HIV infected individual.

36. Ans. (b) 1983 *Ref. Ananthanarayan 8/e, p 570*

History of HIV

- In 1981 AIDS was first recognized when US centre for disease control and prevention reported the unexplain occurrences of P. jiroveci infection in five previously health homosexual male. They appear to have lost their immunocompetence. So the condition was given the name ***"Acquired immune deficiency syndrome"*** (AIDS).
- In 1983 Lue montognier and colleagues from pasteur institute, Paris isolated a retro virus from a West African patient with persistent generalized lymphadenopathy and called it lymphadenopathy associated virus (LAV).
- In 1984 Ader Gallo reported isolation of a retrovirus from AIDS patient and called it human T-cell lymphotrophic virus III.
- To reduce the nomenclature confusion, the intervational committee on virus nomenclature in 1886 decided a generic name ***human immuno deficiency virus*** to the virus isolated from AIDS cases.

Chapter Review

1. **In the heterosexual transmission (from infective partner to non-infective partner) of HIV:** [Kar 03]
   a. There is greater risk of transmission from man to woman
   b. There is greater risk to transmission from woman to man
   c. Risk is equal in neither ways
   d. HIV infection is not transmitted by heterosexual act
   [Ref. Park 22/e, p 320]

2. **In India, maximum cases of tuberculosis in AIDS patients are due to:** [Bihar 03]
   a. *M. tuberculosis* b. *M. avium intracellulare*
   c. *M. scrofulaceum* d. *M. akari*
   [Ref. Ananthanarayan 8/e, p 575, 9/e, p 576]

3. **AIDS involves:** [Jharkhand 04]
   a. T-helper cells b. T-suppressor cells
   c. T-cytotoxic cells d. B-cells
   [Ref. Ananthnarayn 8/e, p 573, 9/e, p 574]

4. **Which of the following gene is associate with encoding of reverse transcriptase?** [Bihar 05]
   a. Pol b. Env
   c. Gag d. p–24
   [Ref. Ananthanarayan 8/e, p 571, 9/e, p 572]

5. **Gag gene encodes for:** [Bihar 05]
   a. Reverse transcriptase b. Core antigen
   c. Envelope d. Gene activation
   [Ref. Ananthanarayan 8/e, p 570, 9/e, p 571]

6. **The most common HIV subtype in India is:** [Kar 06]
   a. D b. H
   c. E d. C
   [Ref. Harrison 19/e, p 1220, 18/e, p 1512 (Fig. 189.7)]

7. **Most common opportunistic infection in AIDS in India:** [Orissa 04]
   a. Toxoplasmosis
   b. Crytococcosis
   c. Cryptosporidosis
   d. TB *[Ref. Ananthanarayan 8/e, p 575, 9/e, p 576]*

8. **Following is the marker of HIV infection in blood:** [AIIMS 94]
   a. Reverse transcriptase
   b. DNA polymerase
   c. RNA polymerase
   d. None *[Ref. Ananthanarayan 8/e, p 580, 9/e, p 581]*

9. **The chances of acquiring HIV infection following needle prick is:** [UPSC 05]
   a. 0.3% b. 3-0%
   c. 0-30% d. 0.003%
   [Ref. Harrison 19/e, p 1222, 18/e, p 1514]

- Risk of HIV infection following skin puncture from a needle or sharp object is 0.3% and after a mucous membrane exposure it is 0.09%.

10. **In a patient having HIV infections, oral ulcer is most commonly due to:** [Orissa 05]
    a. Candida b. Cryptococcosis
    c. Histoplasma d. Trichophyton
    [Ref. Harrison 19/e, p 1255, 18/e, p 1549]

11. **The common causative agent in diarrhea in AIDS patient is:** [UP 07]
    a. Toxoplasma b. Cryptosporidium
    c. Salmonellae d. Mycobacteria
    [Ref. Harrison 17/e, p 1175, 10/e, p 1257]

12. **Which of the following is true about window period of HIV?** [Delhi 08]
    a. PCR is negative
    b. ELISA is positive
    c. ELISA and western blot are positive
    d. Antibody is absent
    [Ref. Ananthanarayan 8/e, p 578, 9/e, p 579]

13. **HIV is not transmitted by:** [Delhi 06]
    a. Sexual contact
    b. Percutaneocus transmission
    c. Transplacental transmission
    d. Body to body contact
    [Ref. Ananthanarayan 8/e, p 581, 9/e, p 582]

14. **T4/T8 cell ratio reversal is seen in:** [RJ 05]
    a. T cell lymphoma b. Hairy cell leukemia
    c. AIDS d. Mumps
    [Ref. Ananthanarayan 8/e, p 575, 9/e, p 574]

15. **HIV is a which type of virus:** [RJ 04]
    a. Picorna virus b. Pox V
    c. Retrovirus d. Herpes
    [Ref. Ananthanarayan 8/e, p 570, 9/e, p 570]

16. **HIV belongs to:** [AP 03]
    a. Lentivirus b. Calcivirus
    c. Retrovirus d. Coronavirus
    [Ref. Ananthanarayan 8/e, p 570, 9/e, p 570]

Answers 1. a. There is greater ... 2. a. *M. tuberculosis* 3. a. T-helper cells 4. a. Pol 5. b. Core antigen
6. d. C 7. d. TB 8. a. Reverse ... 9. a. 0.3% 10. a. Candida
11. b. Cryptosporidium 12. d. Antibody is ... 13. d. Body to ... 14. c. AIDS 15. c. Retrovirus
16. c. Retrovirus

17. Commonest helminthic infection in AIDS is: [UP 00]
a. *Trichuris -Trichiuria* b. *Strongyloides*
c. *Enterobius* d. *Necator americana*
[Ref. Ananthanarayan 8/e, p 576, 9/e, p 576]

18. Following cells are infected by HIV: [Karn. 02]
a. CD8 + T lymphocytes
b. CD4 + T lymphocytes
c. B cells
d. Macrophages *[Ref. Ananthanarayan 9/e, p 574]*

19. Which is not increased in HIV infection?
a. CMV [St John's 02]
b. Kaposi's sarcoma
c. Mycobacterial infection
d. Pneumococcal infection
[Ref. Ananthanarayan 8/e, p 576, 9/e, p 576]

20. Full blown immunodeficiency syndrome is: [SGPGI 03]
a. High viral titres with low CD4 count
b. Low viral titres with low CD4 count
c. Low viral titres with high CD4 count
d. High viral titres with high CD4 count
[Ref. Harrison 17/e, p 1169]

21. All are true about AIDS except: [UP 03]
a. Seen in heterosexual only
b. Caused by retrovirus
c. Candidiasis is also common feature
d. Retrovirus is thermolabile
[Ref. Ananthanarayan 8/e, p 580, 9/e, p 582]

22. Reverse transcriptase is: [UP 04]
a. DNA polymerase
b. DNA dependant RNA polymerase
c. RNA dependant DNA polymerase
d. None *[Ref. Ananthanarayan 8/e, p 570, 9/e, p 571]*

23. Most common mode of transmission of HIV world wide is: [SGPGI 04]
a. Heterosexual
b. Homosexual
c. IV drug abuse
d. Contaminated blood products
[Ref. Harrison 19/e, p 1220, 18/e, p 1510]

24. Screening test for AIDS: [MP 05]
a. ELISA b. PCR
c. Western blot d. CD-4 count
[Ref. Park 22/e, p 323]

25. HIV virus has predilection for infecting: [DNB 05]
a. CD4 + T cells
b. CD8 cells
c. Macrophages
d. Plasma cells
[Ref. Ananthanarayan 9/e, p 574]

26. Most frequent species of Mycobacterirum associated with HIV infection in India is: [MP 06]
a. *M. avium intracelullare*
b. *M. fortuitum*
c. *M. tuberculosis*
d. *M. bovis* *[Ref. Ananthanarayan 8/e, p 575, 9/e, p 576]*

27. Which of the following is not a HIV gene? [Kerala 06]
a. Gag b. Opl
c. Env d. Rb
[Ref. Ananthanarayan 8/e, p 571, 9/e, p 571-572]

28. Antenatal maternal HIV diagnosis is of importance in:
a. To prevent vertical transmission [JIPMER 04]
b. To terminate
c. To discharge
d. To isolate the patient *[Ref. Harrison 19/e, p 1223]*

Combination of antiretroviral therapy along with cesarean section can reduce vertical transmission up to 1%.

29. P24 antigen disappears from the blood after how many weeks infection of HIV: [AMU 05]
a. 2-4 weeks b. 4-6 weeks
c. 6-8 weeks d. 8-10 weeks
[Ref. Ananthanarayan 9/e, p 578]

30. Which infection is not common in HIV patients:
a. Cryptosporidiosis [Kerala 04]
b. Atypical mycobacterial infection
c. Aspergillosis
d. Candidiasis *[Ref. Ananthanarayan 9/e, p 576]*

31. AIDS involves primarily: [AI 98]
a. T-helper cells b. T-suppressor cells
c. T-5 cytotoxic cells d. B-cells
[Ref. Ananthanarayan 8/e p 572, 9/e, p 574]

32. When compared to the Western blot technique, ELISA test is: [AI 96]
a. More sensitive, less specific
b. More sensitive, more specific
c. Less sensitive, less specific
d. Less sensitive, more specific *[Ref. Park 22/e p 323]*

33. All of the following viral genes associated with HIV infection code for structural proteins except: [AI 95]
a. Gag gene b. Env gene
c. Pol gene d. Tat gene
[Ref. Ananthanarayan 8/e p 584, 9/e, p 572]

Answers 17. b. Strongyloides 18. b. CD4 + T ... 19. d. Pneumo ... 20. a. High viral ... 21. a. Seen in ...
22. c. RNA ... 23. a. Heterosexual 24. a. ELISA 25. a. CD4 + T cells 26. c. M. tuberculosis
27. d. Rb 28. a. To prevent ... 29. c. 6-8 weeks 30. c. Aspergillosis 31. a. T-helper...
32. a. More Sens... 33. d. Tate gene

34. In HIV patient hemiparesis, headache, vomiting occur due to infection of which organism: [DNB 2013]
a. Gonococcal inf.
b. Toxoplasmosis
c. Streptococcal
d. None

[Ref. Harrison 18/e 1558]

35. A HIV mother delivers a baby. All are true except:
a. Risk of HIV in the baby is up to 90% [AIIMS 99]
b. HIV infection cannot be diagnosed in the baby with available methods
c. AIDS can be transmitted from mother to child during delivery
d. Breastfeeding can transmit AIDS

[Ref. Harrison 19/e p 1223, 18/e, p 1515]

In the absence of prophylactic antiretroviral treatment the probability of transmission of HIV from mother to fetus ranges from 15 to 25% in developed country and 25 to 35% in developing countries. *..... Harrison, p 1223*

Relative proportion of mother to child transmission are:
23 to 30% – *In utero*
50 to 65% – During birth
12 to 20% – Breastfeeding.

36. Which of the following is not seen in HIV patient with CD4 count less than 100/ml, who has non productive cough? [AIIMS 99]
a. *Mycobacterium tuberculosis*
b. *Pneumocystis carinii*
c. *Mycoplasma pneumoniae*
d. *Cryptococcal infection*

[Ref. Anantharayan 8/e p 576, Harrison 19/e p 1250]

Opportunistic infection in HIV patient when CD4 < 100/µl
- *Coccidiodomycosis*
- *Disseminated MAC*
- *CMV retinitis*
- *Cryptosporidiosis*
- *Histoplasmosis*
- *CVS Lymphoma*

37. Which infection is not common in HIV patients?
a. Cryptosporidiosis [AIIMS 97]
b. *Atypical mycobacterial infection*
c. *Aspergillosis*
d. *Candidiasis*

[Ref. Anantharayan 9/e p 575]

38. Opportunistic infection in HIV patient are: [PGI 96]
a. *P. carinii* b. *M. avium*
c. *Pseudomonas* d. *Mycoplasma*

[Ref. Park 22/e p 321]

39. HIV virus primarily replicates in: [DNB 2012]
a. *CD4 helper T cells*
b. *CD8 T cells*
c. *Natural killer cells*
d. *None*

[Ref. Anantharayan 8/e 572, 9/e p 574]

Answers 34. b. Toxoplasmosis 35. a. Risk of... 36. c. Myco... 37. c. Aspergillosis 38. a, b
39. a. CD4 helper T cells

NEET Pattern Questions

1. **Which of the following is most sensitive for diagnosis of HIV?**
   a. RT PCR
   b. bDNA assay
   c. NASBA
   d. P24 detection
   [Ref. Harrison 18/e, p 1541]

RT PCR can detect as less as 40 copies/ml of HIV RNA, where bDNA assay and NASBA detects when conc. is more than 50 copies/ml and 80 copies/ml respectively.

2. **HIV can infect all except:**
   a. Circulating dendritic cells
   b. CD4 T lymphocytes
   c. Macrophages
   d. Cytotoxic T cells *[Ref. Harrison, 18/e, p 1528]*

3. **Co-receptor for R5 variant of HIV virus:**
   a. Integrin
   b. CCR 5
   c. CXCR 4
   d. P 53
   [Ref. Harrison 18/e, p 1528]

4. **An HIV patient developed goitre. On examination there was non-tender diffuse enlargement of thyroid. All of the following can be cause of it, except:**
   a. Toxoplasma
   b. Cryptococcus
   c. Aspergillus
   d. Pneumocystic
   [Ref. Harrison 18/e, 1554]

In an HIV patient thyroid functions may be altered in 10-15% patients. Both hypo and hyperthyroidism may be seen though the former is more common.
In advance HIV disease infection of thyroid may occur with opportunistic pathogens including P. jiroveci, CMV, mycobacteria, Toxoplasma gondii and (ryptococcus neoformans.

5. **Binding of gp 120 causes:**
   a. Infection of target cell
   b. Facilitation of co-receptor
   c. Fusing of virus and target cell
   d. None *[Ref. Harrison, 18/e, p 1507]*

The replication cycle of HIV begins with the high affinity bindings of the gp 120 protein to CD4 molecule on host cell. After binding gp 120 protein undergoes confirmational changes that facilitate binding of one of two major co-receptor (CCR-5. CXCR4).

6. **What is p24:**
   a. Envelope antigen in HIV
   b. Core antigen in HIV
   c. Genome of HIV
   d. Shell antigen *[Ref. Harrison, 9/e, p 572]*

7. **Nef gene in HIV is for use:**
   a. Enhancing the expression of genes
   b. Enhancing viral replication
   c. Decreasing viral replication
   d. Maturation *[Ref. Harrison, 9/e, p 573]*

8. **Most sensitive test for HIV infection:**
   a. Western blot
   b. ELISA
   c. Agglutination test
   d. CFT
   [Ref. Harrison, 22/e, p 323]

9. **HTLV extra gene is:**
   a. Gag
   b. Pol
   c. Env
   d. Tex

Some retrovirus such as HTLV and HIV carry a fourth gene tex or tat after env gene. This is a transactivating gene that regulates the function of viral gene.

10. **The chance that a health worker gets HIV from an accidental needle prick is:**
    a. 1%
    b. 10%
    c. 95%
    d. 100%
    [Ref. Harrison, 19/e, p 1222]

HIV transmission following skin puncture from a needle or a sharp object that was contaminated with blood from a person with documented HIV infection is 0.3% and after a mucous membrane exposure is 0.09%

11. **Most common opportunistic infection in HIV, globally:**
    a. P. jiroveci
    b. Candida
    c. M. tuberculosis
    d. Cryptococcus
    [Ref. Park, 22/e, p 318]

12. **HIV envelop is formed by:**
    a. Host cell
    b. Virus
    c. Both
    d. None
    [Ref. Ananthanarayan, 9/e, p 571]

Lipoprotein Envelop of HIV virus: When naked virus buds out of cell surface it acquire a lipoprotein envelope which consists of lipid derived from the host cell membrane and glycoprotein which are virus coded.

Answers 1. a. RT PCR 2. d. Cytotoxic T cells 3. b. CCR5 4. c. Aspergillus 5. b. Facilitation of...
6. b. Core anthigen... 7. c. Decreasing viral... 8. a. Western blot 9. d. Tex 10. a. 1%
11. c. M. tuberculosis 12. c. Both

13. **Which HIV-virus is more dangerous:**
    a. HIV-1
    b. HIV-2
    c. Both are same
    d. It depends on host factors
    [Ref. Ananthanarayan, 9/e, p 573]

HIV-1 is more virulent. HIV-2 has only 40% genetic identity with HIV-1. It is more closely related to simian immunodeficiency virus.

14. **Function of reverse transcriptase:**
    a. RNA dependent DNA synthesis
    b. DNA dependent RNA synthesis
    c. DNA dependent DNA synthesis
    d. RNA dependent RNA synthesis
    [Ref. Greenwood, 18/e, p 566]

15. **A person has unprotected sex 3 weeks back. To rule out HIV infection the best test is:**
    a. P24 antigen assay *[Ref. Ananthanarayan, 9/e, p 576]*
    b. ELISA
    c. Western blot
    d. Lymphnode biopsy

For details see answer no. 11 in explanatory answers

16. **HTLV-1 is also known as:**
    a. HIV
    b. ATLV
    c. RSV
    d. ALV
    [Ref. Ananthanarayan, 9/e, p 575 (See answer 11 pNo. 380)]

17. **Most common mode of transmission of HIV sexual transmission:**
    a. Blood & blood products
    b. Occupational
    c. Perinatal
    d. Breast feeding

Answers **13.** **a.** HIV-1 **14.** **a.** RNA dependent DNA synthesis **15.** **a.** P24 antigen assay
16. **b.** ATLV **17.** **a.** Blood & blood products

Section - B

UNIT – III Mycology

- Dermatophytes
- Yeast and Yeast-like Fungus
- Aspergillus and Mucormycosis
- Dimorphic Fungi

CHAPTER

29

Dermatophytes

- Filamentous fungi (Mould) that infect only superficial keratinized tissues – skin, hair and nail.
- Causative agent of *Ringworm or Tinea or Dermatophytoses.*
- Not involve living tissues.
- ***It classified in to 3 genera*** on the base of morphology of macroconidia – (*Trichophyton, Microsporum, Epidermophyton).*
- ***In lesion,*** it form *hyphae* and *arthospores.*
- ***In culture*** it form *septate hyphae* and *asexual* spores (micro and macroconidia) with powdery and pigmented colonies.
- They are *differentiated* mainly by *nature of macrocondia.*
- In some species of dermatophytes sexual reproductive state has been discovered and all dermatophyte with a sexual form belong to telemorphic genus *Arthroderma.*
- *Culture media: Sabauraud's agar*

I

Dermatophytes:
Infect keratinised tissue only.
Includes:
- Trichophyton: Infect hair, skin and nail
- Microsporum: Infect hair and skin
- Epidermophyton: Infect skin and nail only.

Clinical Features

- Local inflammation is due to irritation by fungal products and hypersensitivity reaction.
- Transmission occurs from *infected to uninfected person often by brushes, combs and towels.*

Features	Trichophyton	Microsporum	Epidermophyton
Site	Infect hair, skin and nail	Hair and skin only	Skin and nail only
Mnemonic	*Trishna*	*Mi skin hairs*	*Epi ski nai*
Colony	Powdery, pigmented	Cotton like pigmented	Powdery greenish yellow
Spores			
• Microconidia	Abundant	Relatively scanty	Absent
• Macroconidia	Pencil or Cylindrical shaped, relatively scanty	Multicellular Spindle or fusiform shaped and is predominant spore	Club-shaped or pear-shaped multicellular

- Clinically ringworm is classified depending on the site involved, e.g. *Tinea capitis* infect scalp and hair.
- *MC* **species** infecting human being – ***T. rubrum.***

> **Note:** ***Dermatomycosis: Sometime used as synonym of dermatophytoses, truely it includes the cutaneous manifestation of other systemic mycosis also (e.g. Candida)***

Lab Diagnosis

- ***KOH mounts :*** Scrapings from edges of ringworm lesions + 10% KOH. Microscopy reveals branched septate hypae.
- ***UV light (wood's lamp)*** : To facilitate selection of infected hair for examination (*infected hair appears fluorescent*). Two types of hair infection may be seen:
  - **Ectothrix** - Arthrospores form sheath around the hair.
  - **Endothrix** - Spores are inside the hair shaft.
- ***Culture***: Sabouraud's medium (with antibiotics and cycloheximide)

Treatment

- Topical antifungal agents, Oral griseofulvin is DOC.

Multiple Choice Questions

1. ***T. capitis* (endothrix) is caused by:** [PGI 00]
   a. *Epidermophyton*
   b. *T. tonsurans*
   c. *T. violaceum*
   d. Microsporum

2. **Kerion is caused by:** [PGI 98]
   a. *Candida* b. *Streptococcus*
   c. Dermatophytes d. Herpes

3. ***Tinea cruris* is caused by:** [PGI 97]
   a. *Epidermophyton* b. *Trichophyton*
   c. *Microsporum* d. *Candida*

Explanations and References with Illustrative Answers

1. **Ans. (b) and (c) *T. tonsurans* and *T. violaceum*** *Ref. Ananthnarayan 8/e, p 606, 9/e, p 597*

Dermatophytoses	Common causative agents
Tinea capitis	Microsporum, Trichophyton most species
Favus	*T. schoenleinii T. violaceum, M. gypseum*
Tinea barbae	*T. rubrum, T. mentagrophytes, T. verrucosum*
Tinea imbricata	*T. concentricum*
Tinea corporis	*T. rubrum* and any other dermatophyte
T. cruris (Tock itch)	*E. floccosum, T. rubrum*
T. pedis (Athlete foot)	*T. rubrum, E. floccosum*
Ectothrix hair infection	Microsporum species, *T. rubrum*
Endothrix hair infection	*T. schoenleinii, T. tonsurans, T. violaceum*

2. **Ans. (c) Dermatophytes** *Ref. Dashore Manual of Skin, p 26*

Kerion
- The fungal infection of scalp caused by *microsporum or trichophyton* species.
- Inflammatory boggy swelling covering small or large areas of scalp in which hair are loose and fallout or can be easily epilated.
- Commonly caused by zoophilic dermatophytes like *T. mentagrophytes* and *T. verrucosum.*
- Follicular scarring and partial alopecia is common after severe kerion.

Favus
- Chronic type of ringworm in which dense crusts (scutula) develop in hair follicles leading to alopecia and scarring

Remember: Both kerion and favus are type of *Tinea captis.*

3. **Ans. (a) and (b) Epidermophyton and Trichophyton** *Ref. Ananthnarayan 8/e, p 606, 9/e, p 597*

Already explained, refer Ans. 1

Chapter Review

1. **The causative agent of Favus is:** [COMED 08]
   a. *Microsporum audonii*
   b. *Microsporum canis*
   c. *Trichophyton mentagrophyte*
   d. *Trichophyton schoenleinii*

   [Ref. Ananthanarayan 8/e, p 606, 9/e, p 597]

2. **Dermatophytosis is caused by:** [Delhi 02]
   a. Herpes simplex b. Papilloma virus
   c. *Trichophyton* d. *Candida*

   [Ref. Ananthanarayan 8/e, p 604, 9/e, p 596]

3. **Back dot ringworm is caused by:** [UPSC 04, PGI 79, AIIMS 85]
   a. *Microsporon* b. *Trichophyton*
   c. *Epidermophyton* d. *Candida*

   [Ref. Jawetz 24/e, p 629]

4. **Pityriasis versicolor is caused by:** [UP 07]
   a. *E. floccosum* b. *M. gypseum*
   c. *M. furfur* d. *T. tansurans*

   [Ref. Ananthanarayan 8/e, p 603, 9/e, p 595]

Pityriasis versicolor

- Chronic asymptomatic involvement of the stratum corneum characterised by discrete or confluent macular areas of discoloration or depigmentation
- Causative agent is a lipolytic, yeast-like fungus pityrosporum orbicular (Malassezia furfur)

5. **Tinea pedis is caused by which of the following:** [MAHA 05]
   a. *E. floccosum* b. *M. furfur*
   c. *M. canis* d. *E. werneckii*

   [Ref. Ananthanarayan 7/e, p 614, Jawetz 24/e, p 628]

Tinea pedis is most prevalent of all dermatophytoses and usually occur as chronic infection of toe webs. It is caused by *T. rubrun* and *E. floccosum*.

6. **The organism that does not affects hair:** [NEET/APPGE 05]
   a. Trichophyton rubrum
   b. Epidermiphyton floccosum
   c. Microsporum
   d. All

   [Ref. Ananthanarayan 8/e, p 605, 9/e, p 599]

7. **Trichophyton infects:** [NEET/DNB 09]
   a. Skin b. Hair
   c. Nail d. All

   [Ref. Ananthanarayan 8/e, p 604, 9/e, p 599]

Answers 1. d. Trichophyton... 2. c. Trichophyton 3. b. Trichophyton 4. c. M. furfur 5. a. E. floccosum 6. b. Epidermiphyton... 7. d. All

NEET Pattern Questions

1. **Dermatophytids are:**
   a. Fungal hyphae in skin
   b. Vegitative fungal cells in keratinised tissue
   c. Cutaneous lesions secondary to hypersensitivity to fungal antigens
   d. Dead fungal tissue

[Ref. Ananthanarayan 9/e, p 597]

Dermatophytids are the cutaneous lesions secondary to hypersensitivity to fungal antigens. The reaction may follow oral antifungal therapy

2. **Regarding fungal cell wall all are true except:**
   a. Contains chitin
   b. Prevent osmotic damage
   c. Azoles act on them
   d. Does not contain peptidoglycan

[Ref. Book of General Microbiology 2/e, p 58]

Fungi possess rigid cell wall which is composed polysaccharide homopolymers such as glucans and chitin, rather than peptidoglycan (a component of bacterial all wall)

- Functions of fungal cell wall
  - Gives shape and farm
  - Protects against mechanical injury
  - Protects against osmotic lysin
- Drugs acting on fungal cell wall: Amphotericin and Nystatin, Hamycin, Natamycin, Caspofungin.
- Azoles inhibit ergosterol synthesis and interfere membrane function

3. **Trichophyton species which is zoophillic:**
   a. T. tonsurans
   b. T. violaceum
   c. T. schoenleinii
   d. T. mentagrophytes

[Ref. Harrison 18/e, p 47]

Zoophilic trichophyton are T. montegraphytes and T. vemcosum

Note: Zoophilic dermatophytes are the species which principals infects animals and only occasionally infect human.

4. **Sclerotic bodies are associated with:**
   a. Mycetoma
   b. Chromoblastomycosis
   c. Sporotrichosis
   d. Rhinosporidiosis *[Ref. Ananthanarayan 9/e, p 601]*

Chromoblastomycosis

- Most common form of chromomycosis
- Lesion consist of warty, cutaneous nodules which resemble the florets of a cauliflower
- Most common fungi responsible are species of the genera Fonsecaea and Cladophialophora
- Histologically the lesion show the presence of the fungus as round or irregular dark brown yeast like bodies with septae called sclerotic bodies.

Answers 1. c. Cutaneous lesions... 2. c. Azoles act on them 3. d. T. mentagrophytes 4. b. Chromoblastomycosis

CHAPTER

30

Yeast and Yeast-like Fungus

CRYPTOCOCCUS NEOFORMANS

- Only yeast which is pathogenic (Infact most life threatining fungal pathogen). Only deep mycosis which is common in India. Also called as European blastomycosis.
- Characterized by a thick polysaccharide capsule which can be stained by India Ink.
  ..... *Ananthanarayan 9/e, p 613*
- There are two species, *C. neoformans* and *C. gattii* which can cause cryptococcosis in humans. *C. gattii* to rare and may infect normal individual.
- *C. neoformans* is found in feces of pigeon and other birds where as *C. gatti* inhabits eucalyptus tree.
- *C. neoformans* occurs in two varieties *grubii* and *neoformans* which correlates with *serotype A* and *D respectively*.

I

Cryptococcus:
- Only pathogenic yeast and is characterized by thick polysaccharide capsule.
- Pathogenic cryptococcus grows at 37°C whereas others do not
- Most common predisposing factor: AIDS
- Meningoencephalitis is the most important manifestation

Morphology

- **Cell wall** is Gram-positive.
- All species of cryptococcus are encapsulated and possess urease.
- *C. neoformans differs* from nonpathogenic species of *Cryptococcus* by its ability to grow at 37°C and the production of laccase, a phenol oxidase which catalyses formation of melanin, and its ability to produce urease.
  ..... *Jawetz 27/e, p 688*
- Capsule and laccase are virulence factors.

I

- Commonest site of clinical manifestation: CNS
- Primary site of infection: Lungs

Pathogenesis and Pathology:

- *MC predisposing factor* for cryptococcosis: **AIDS** (risk increase when CD4 <200/ul).
- Most infections in immunocompromised patient are caused by serotype A.
- Infection is acquired by inhalation of the fungus into lung, which is frequently asymptomatic. However, in immunocompromised state there is hematogenous spread from lung to brain which leads to meningoencephalitis.
- Cryptococci are best seen in tissue by staining with *methenamine silver or periodic acid* schiff.
- Infection can occur by pigeon dropping (Containing serotypes A & D) or eucalyptus tree (Containing serotype B).

I

- In addition to their capsular serotypes, the two species differ in their genotypes, ecology.

Clinical Manifestation

- Most patient present with meningoencephalitis, though primary site of infection is lung.
- *C. neoformis* is *MC* cause of *meningitis in* **AIDS** patient.
- *C. meningitis* may not show meningismus, which is seen in bacterial meningitis and it may be associated with sudden vision loss.
- In crypococcal meningitis CSF examination reveals evidence of chronic meningitis with mononuclear pleocytosis and increased protein level and reduced glucose level.
- Focal lesions called cryptococcomas are *more common* in previously normal patient.
- These lesions are located *MC* at **basal ganglia** or the head of caudate nucleus.
- Cryptococcomas are *best* seen with *MRI.*
- *Cryptococcus* in **AIDS** patient has less sign and symptoms.
- Headache is *MC* symptom.
- Uncommon manifestation of cryptococcus include, skin lesions resembling mollascum contagiosum, lymphadenopathy, palatal and glossal ulcers, arthritis, prostitis, myocarditis.

Diagnosis

- Lumbar puncture is single most useful ***diagnostic test.*** Visualization of capsules of fungal cells in CSF is a useful rapid diagnostic technique.
- *Indian Ink preparation* is the *method of choice for detecting cryptococci in CSF.*
- Culture of CSF and blood for C. neoformans
- *Latex agglutination* – Approx 90% of patient with cryptococcal meningitis including all AIDS patient, have capsular antigen detectable in CSF. However this is less useful in diagnosis of pulmonary disease.

Treatment

Type of disease		Preferred treatment	Alternative
i.	Disease in AIDS Patient	Amphotericin B	Itraconazole
ii.	Disease in non AIDS Patient		
	– Meningitis	Amphotericin B	Switch over to fluconazole when patient condition has improved
	– Pulmonary disease		
	Immunocompromised Patient	Amphotericin B	Switch over to fluconazole when patient condition has improved
	Normal	Fluconazole	Itraconazole

CANDIDA

Candidiasis is *MC* systemic mycosis.

- *MC* agent responsible is *Candida albicans.*
- All pathogenic *Candida* are commensals of humans particularly in mouth, stool and vagina.

I

Candida

- Most common systemic mycosis
- Candida albicans is the most common pathogenic candida
- Candida albicans form chlamydospores at 20°C on corn meal agar
- Diabetes is the most common risk factor.

Morphology

- Gram-positive fungi
- They grow as budding yeast cells but they can also form pseudohypahe (except C. *glabrata* which does not) both in culture and in tissues.
- *Candida albicans* is dimorphic as it also forms true hyphae *[other species of Candida are not dimorphic].* ... *Jawetz 27/e, p 684*
- *C. albicans* is identified on basis of their ability to form of large thick walled spores *(diagnostic)* called *chlamydospores* on nutritionally deficient media or corn meal agar at 20°C.
- *C. albicans* form germ tubes *[Reynolds Braude Phenomenon].*
- *Accurate identification* of *Candida* species other than C. *albicans* require biochemical tests.

Pathogenesis

- Invasive candidiasis is usually preceded by ↑ colonization in mouth, vagina due to broad spectrum antibiotics.
- Majority of non-albicans vagina species enter the blood through intravascular catheterization.

Clinical Manifestation

I. *Cutaneous and mucosal candidiasis:*
- **Risk factors** association with superficial candidiasis are:
  - AIDS
  - Infants and elders
  - Steroids
  - Pregnancy
  - Oral contraceptives
  - **Diabetes** *(MC)*
  - Trauma

It includes the following

A. Oral thrush
- Painless discrete and confluent adherent white plaques on the oral and pharyngeal mucosa, common in AIDS at CD4 <50/µl, bottle fed infants, debilitated patient.
- Cutaneous candidiasis may be **intertriginous** (erythematous scaling or moist lesion) or **paronychial** (seen in occupation which require frequent immersion of hands in water).

B. Esophageal candidiasis:
- Most lesion are in distal 3rd of esophagus. Diagnosed by biopsy.

C. Vulvovaginal candidiasis:
- Mostly in 3rd trimester of pregnancy.

D. Chronic mucocutaneous candidiasis:
- Mostly onset is in early childhood and association with cellular immunodeficiencies and endocrinopathy. Present as hyperkeratotic skin lesion, partial alopecia, and both oral and vaginal thrush.

II. ***Deeply Invasive (systemic) candidiasis*:**

Risk factor for invasive candidiasis
- *Prolonged neutropenia*
- *Broad spectrum antibiotic therapy*
- *Intravenous drug use*
- *Recent surgery*
- *Presence of intravascular catheters*

- Hematogenous seeding (candidemia) is common to retina, kidney, spleen, liver.
- ***In immunocompetent patient***– U/L or B/L white retinal exudates appear within 2 weeks of onset of candidemia. Most cases of ocular involvement occur in non neutropenic patient.
- ***Hepatosplenic candidiasis*** (Chronic disseminated candidiasis) usually occur in patient with acute leukemia. originates from intestinal seeding of portal and venous circulation. Mostly occur in neutropenic patient.
- *Candida* can cause arthritis of knee in patient who have received *chronic glucocorticoid* injections in joint.
- Endocarditis in previously damaged or prosthetic valve.

I
- Diabetes is the most common risk factor for mucocutaneous candidiasis
- Neutropenia is the most prominent risk factors for invasive candidiasis

Diagnosis

i. ***Superficial candidiasis*:** Demonstration of pseudohyphae or hyphae on wet smear with confirmation by culture or staining (Gram's, PAS, Methenamine silver).

ii. ***Invasive candidiasis*:** Diagnosed by histologic section of biopsies or by culture of CSF, blood, joint fluid. C. albicans is identified by production of germ tubes or chlamydospores.
- A rapid method for identifying Candida albicans is based on its ability to form germ tubes within two hours when incubated in human serum at 37°C *(Reynolds–Braude Phenomenon)*

Treatment

Type			Preferred	Alternative
i.	***Mucocutaneous***			
	•	Cutaneous	Topical azoles	Topical nystatin
	•	Vulvovaginal	Azole cream or oral fluconazole	Nystatin suppository
	•	Oropharyngeal	Clotrimazole or fluconazole	Nystatin
	•	Esophageal	Fluconazole or itraconazole	
ii.	***Deeply invasive***			
	•	Non-neutropenic	Fluconazole or amphotericin B	
	•	Neutropenic	Amphotericin B	
iii.	***Candida* endophthalmitis**		IV polyene + flucytosine	

PNEUMOCYSTIS INFECTION

I

Pneumocystis
P. jiroveci is the human isolate
- Major surface glycoprotein in the most important antigen
- AIDS is the most common predisposing factor
- PCR is the most sensitive technique for diagnosis.

- Opportunistic fungal pulmonary pathogen, lacks ergosterol so not susceptible to antifungal which inhibit ergosterol synthesis.
- Human isolate – *P. jiroveci; P. carinii* is found in rats. *... Harrison 18/e, p 1671*
- **Most prominent antigen**: Major surface glycoprotein which shows antigenic variation and facilitates its adherence.
- Other important antigen is 35-55kDa - which acts as marker of infection.
- In tissues P. Jiroveci occur in two distinct forms:
  - Thin walled trophozoites.
  - Thick walled spherical or elliptical cysts which contain four to eight nuclei. Cyst can be stained with silver stain, toulidine blue, calcoflour white.

Pathogenesis and Pathology

I

Pneumocystic pneumonia
- Transmission: Air borne, person to person
- CXR: Bilateral diffuse perihilar infiltrates

- Defects in CMI and humoral immunity predispose for its reactivation or reinfection, e.g. In HIV infection occur when ***CD4 + T cell count <200/µl.***
- Principal host effector cells – Alveolar macrophage.
- ***Transmission*** – Airborne; person-to-person.
- **In alveoli:**
  - It attaches to **type I cells** (extracellular) and damages it.
  - Hypertrophy of type II cells occur, causing surfactant abnormalities.
  - Increase IL-8 and neutrophil in BAL fluid.
  - Alveoli gets filled with typical foamy vacuolated exudate.
- **Malnourished infants exhibit an intense** plasma cell interstitial infiltrate seen (so earlier called as *Interstitial plasma cell pneumoniae).*

Risk Factors for *Pneumocystis pneumonia*
- HIV
- Immunosuppressive therapy particularly glucocorticoids for cancer
- Organ transplantation
- Children with primary immunodeficiency disease and premature malnourished infants.

I

P-carinii produce interstitial pneumonia

Clinical Feature

- **Symptom** – Dyspnea, fever, non-productive cough.
- **Sign**– Tachypnea tachycardia and cyanosis but lung auscultation reveals few abnormalities.
- Risk for extrapulmonary spread increase with: Administration of aerosolized pentamidine.
- *MC* extrapulmonary site: **Lymph node**, spleen, liver, bone marrow.
- Most widely used prognostic factor is degree of hypoxemia.

Diagnosis

1. **Definitive diagnosis by histopathologic detection by:**
   a. Staining – Methenamine silver, toludine blue stain cell wall while *Wright-Giemsa stain the nuceli.*
      – Immunoflurescence with monoclonal antibodies; more sensitive.
   b. DNA amplification by PCR – *most sensitive.*
2. **Specimen collection**
   a. ***Fiberoptic bronchoscopy with BAL*** (which is more sensitive than sputum induction) is the mainstay of diagnosis.
   b. ***Transbronchial biopsy and open lung biopsy*** – *only when* diagnosis cannot be made by BAL.
3. **Chest X-ray**
   - Classic findings include
     – B/L diffuse infiltrate in perihilar region.
     – Nodular densities, cavitary lesion, Pneumothorax can also occur.
     – ↑*Frequency of upper lobe infiltrate in patient who take aerosolized pentamidine.*

Treatment

- *DOC* ***cotrimaxazole*** for all forms of pneumocystosis including extrapulmonary disease.
- Alternative for mild to moderate case – Trimethoprim + dapsone and clindamycin + primaquine.
- Alternative for moderate to severe – Pentamidine slow IV.
- Adjunctive therapy–Glucocorticoid in HIV patient with moderate to severe pneumocystosis whose pulmonary function deteriorates on taking anti-pneumocystis drugs.

Prophylaxis

- *DOC* for HIV primary and secondary (both HIV and Non HIV) prophylaxis is cotrimoxazole.
- Alternative Dapsone
- In HIV prophylaxis is indicated when:
  – CD_4 + <200/µl
  – History of oropharyngeal candidiasis.

Multiple Choice Questions

Cryptococcus

1. **The capsule of *Cryptococcus neoformans* in a CSF sample is best seen by:** [AI 05]
   a. Gram's stain
   b. Indian ink preparation
   c. Giemsa stain
   d. Methanamine-silver stain

2. **The most common organism amongst the following that cause acute meningitis in an AIDS patient is:** [AI 05]
   a. *Streptococcus pneumoniae*
   b. *Streptococcus agalactiae*
   c. *Cryptococcus neoformans*
   d. *Listeria monocytogenes*

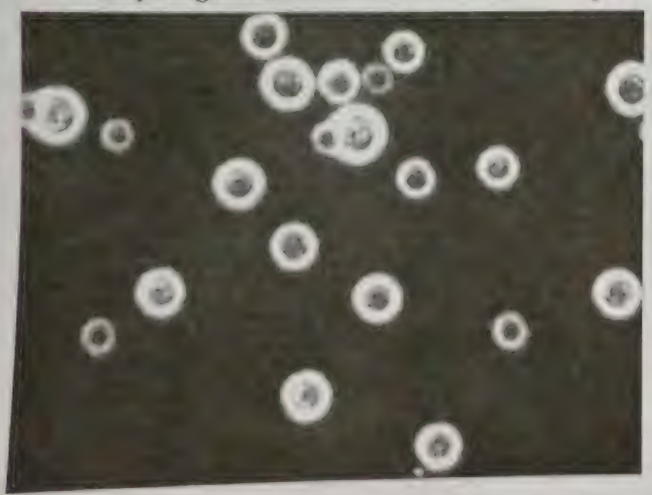
3. **Latex agglutination test of the antigen in CSF helps in the diagnosis of:** [AI 00]
   a. *Cryptococcus*
   b. *Candidiasis*
   c. *Aspergillosis*
   d. *Histoplasmosis*

4. **Cryptoccoccal meningitis is common in:** [PGI 00]
   a. Renal transplant recipient
   b. Agammaglobulinemia
   c. Neutropenia
   d. IgA deficiency

5. **Cryptococcus can be readily demonstrated by:**
   a. Albert's stain [PGI 02]
   b. India ink stain
   c. Giemsa's stain
   d. Gram's stain
   e. Z-N stain

6 (a). **Neurotrophic fungus is/are:** [PGI 02]
   a. *Cryptococcus neoformans*
   b. Histoplasmosis
   c. *Trichophyton*
   d. *Candida*
   e. Aspergillosis

6 (b). **A patient presented with headache and projectile vomiting along with alteration in sensorium. The following parasite demonstrated on India ink staining. What is the likely diagnosis:** [PGI 02]



   a. Cryptococcus
   b. Blastomyces
   c. Histoplasma
   d. Coccidiodes

Candida

7. **An HIV positive female has an indurated ulcer over the tongue. Laboratory findings show growth in cornmeal agar at 20 degrees, microscopy showing hyphae and growth inhuman serum at 37 degrees show budding yeasts. The probable cause is:** [PGI 06]
   a. *Candida albicans*
   b. *Histoplasmosis*
   c. *Blastomycosis*
   d. *Coccidiodomycosis*
   e. *Mucormycosis*

8. **Most common fungal infection in febrile neutropenia is:** [AI 01]
   a. Aspergillus niger
   b. *Candida*
   c. Mucormycosis
   d. Aspergillus fumigatus

9. **HIV positive female presents with an indurated ulcer over the tongue. Laboratory findings show growth in cornmeal agar at 20°C, microscope by showing hyphae and growth in human serum at 37°C show budding yeast. Probable cause is:** [AIIMS 01]
   a. *Candida albicans*
   b. *Histoplasmosis*
   c. *Blastomycosis*
   d. *Coccidioidomycosis*

10. **(i) Which among the following is the most common fungal infection seen in immunocompetent patients:** [AIIMS 10, AI 2011]
   a. Aspergillus
   b. Cryptococcus
   c. Candida
   d. Penicillium marneffei

10. **(ii) The infection that is spreading to the newborn by caregivers?** [AIIMS May 2014]
   a. Candida albicans
   b. Candia galbrata
   c. Candida parapsilosis
   d. Candida tropicans

Pneumocystis

11. ***Pneumocystis Jeroveci*:** [AI 08]
   a. Associated with CMV
   b. Diagnosis is by sputum microscopy
   c. Seen only in immunocompromised patients
   d. Always associated with pneumatocele

12. ***Pneumocystis carinii* is a fungus because:** [PGI 00]
   a. rRNA, mitochondrial protein gene sequence and presence thymidylate late synthase
   b. Cell wall contains glucans
   c. Antifungals are effective against *P. carinii*
   d. Commonest infection in AIDS

Others

13. **Which dye is most suitable for fungus demonstration in biopsy:** [AIIMS 06]
   a. Alizharian red
   b. Verihoff dye
   c. Mason's trichome
   d. PAS

14. **In HIV infected individual Gram stain of lung aspirate shows yeast like morphology. All of the following are the most likely diagnosis except:** [AIIMS 05]
    a. *Candida tropicalis*
    b. *Cryptococcus neoformans*
    c. *Penicillium marneffi*
    d. *Aspergillus fumigates*

15. **All are yeast like fungus except:** [AIIMS 97, PGI 06]
    a. *Cryptococcus* b. *Candida*
    c. *Trichophyton* d. None of the above

16. **Endemic fungal infection is caused is by all of the following except:** [PGI 05]
    a. *Coccoides immitus* b. *Cryptococcus*
    c. *Penicillium* d. *Aspergillus*
    e. *Blastomyces*

17. **Which of the following are difficult to isolate from culture:** [PGI 03]
    a. *Candida* b. *Dermatophytes*
    c. *Cryptococcus* d. *Malassezia furfur*
    e. *Coccidiodomycosis*

18. **Fungi without sexual cycle are classified as:** [PGI 2000, 97]
    a. Fungi imperfecti b. Phycomyces
    c. Ascomycetes d. Basidiomycetes

19. **Maltese cross in polarizing microscopy is seen in:** [AIIMS 10]
    a. Cryptococcus neoformans
    b. Penicillium marneffi
    c. Blastomyces
    d. Candida albicans

Explanations and References with Illustrative Answers

1. **Ans. (b) Indian Ink preparation** *Ref. Ananthanarayan 9/e, p 613; 8/e, p 610; Harrison 19/e, 1339, 18/e, 1650*
   - *Cryptococcus neoformans* is the only pathogenic yeast.
   - Within the host and on certain culture media, it is surrounded by a large polysaccharide capsule.
   - Capsule stands out in ***India Ink preparation.*** *... Ananthanarayan 8/e, p 610*
   - ***Indian Ink smears remains the method of choice for detecting Cryptococcus in "CSF".*** *... Harrison 19/e, p 1340*
   - Cryptococci are best seen in tissue by staining with methamine silver or periodic acid schiff (They stain the fungus itself not capsule).
   - Muciramine staining of tissue is diagnostic but demonstrable only in few cases.

2. **Ans. (c) *Cryptococcus neoformans*** *Ref. Harrison 19/e, p 1346, 18/e, p 1649 - 1650*
   - *C. neoformans* is *MC* cause of meningitis in AIDS patient.
   - Generally occurs when CD4+ T cell count **<100/μl.**
   - Diagnosis is made by identification of *C. neoformans* by India Ink or by detection of cryptococcal antigen by latex agglutination test.
   - Strongly +ve result on muciramine staining of tissue is ***diagnostic.***

   Other important features of cryptococcal meningitis:
   - *MC* cause of meningitis in AIDS patient
   - Predisposing factors: - HIV infection - Solid organ transplantation - Hodgkins disease
     - Sarcoidosis - Corticosteroid therapy

   Treatment: Patient with AIDS and high risk patient: I.V Amphotericin B followed by fluconazole for maintainance.

3. **Ans. (a) *Cryptococcus*** *Ref. Harrison 16/e, p 1184, 18/e, p 1650; 19/e,p 1340, CMDT '2014 p 1485*

"90% of patient with cryptococcal meningoencephalitis have capsular antigen detectable in CSF or serum by latex agglutination."

... Harrison 16/e, p 1184

Diagnosis of cryptococcal disease					
Meningoencephalitis		***Pulmonary Cryptococcus***		***Cutaneous Cryptococcus***	
–	Lumbar puncture is **most useful** test	–	Mimics malignancy	–	Biopsy
–	India ink smear of CSF reveals encapsulated yeast in more than half of cases	–	Biopsy is required for diagnosis		
–	90% of patient have capsular antigen detectable in CSF by latex agglutination				
–	CSF culture is definitive diagnostic test				

Note: MRI is more sensitive than CT in finding central nervous system cryptococcomas. *... CMDT 2014 p1485*

4. **Ans. (a) Renal Transplant recipient** *Ref. Harrison 19/e, p 1339, 18/e, p 1649*

Predisposing factors of *Cryptococcus*	
• AIDS	• Hodgkin' Hematological malignancy
• *Solid organ transplant recipient*	• *Cortiocosteroid therapy*
• *Sarcoidosis.*	

Note: Among HIV(+)ve individuals, those with a decreased percentage of B-cells expressing IgM may be at a greater risk for cryptococcosis.

5. **Ans. (b) Indian Ink Stain** *Ref. Harrison 19/e, p 1338, 18/e, p 1650*

Already explained

6(a). **Ans. (a), (b), (d) and (e) *Cryptococcus neoformans; Histoplasmosis; Candida;* and Aspergillosis** *Ref. Harrison 16/e, p 2492*

Fungus causing CNS infection:
- *Cryptococcus*
- *Histoplasmosis*
- Sporothrix schenckii
- *Coccidiodomycosis*
- *Blastomycosis*
- *Aspergillus*
- *Candidiasis*

6(b). **Ans. (a) Cryptococcus** *Ref. Jawetz 27/e, p 687*

This is a characteristic image of cryptococcus capsule when stained under India ink.

7. **Ans. (a) *Candida albicans*** *Ref. Harrison 19/e, p 1342, 18/e, p 1652*

"This is a case of oral thrush secondary to candidiasis"

- *C. albicans* is a dimorphic fungi which occur both as yeast and moulds (with hyphae).
- In HIV oral thrush occurs when CD4 <50/µl.

8. **Ans. (b) *Candida*** *Ref. CMDT 2014, p 1233, 492*

Neutropenia ↑ chance of following infection.

Bacterial	***Fungal***	***Viral***
Gram – ve enteric pathogens ***(MC)***	*Candida* ***(MC)***	Herpes zoster
Pseudomonas	*Aspergillus*	CMV
Gram +ve cocci (particularly *Staph. aureus; Staph epidermidis*, and viridans streptococci)	*Pneumocystis carnii*	Respiratory syncytial virus Influenza virus

Organism cause infection in granulocytopenic patients *Ref. Harrison 17/e, p 535*

- ***Gram-positive cocci***
  - *Staphylococcus epidermidis*
  - *Staphylococcus aureus*
  - Viridans *Streptococcus*
  - *Enterococcus faecalis*
  - *Streptococcus pneumoniae*
- ***Gram-positive bacilli***
  - *Diphtheroids*
  - JK bacillus
- ***Gram-negative bacilli***
  - *Escherichia coli*
  - *Klebsiella spp.*
  - Non-aeruginosa *Pseudomonas spp.*
  - *Enterobacter* spp.
  - *Serratia spp.*
  - *Acinetobacter* spp.
  - *Citrobacter* spp.
- ***Fungi***
  - *Candida* spp.

9. Ans. (a) *Candida albicans* *Ref. Harrison 19/e, p 1342; 18/e, p 1652*

This is a case of oral thrush due to Candida albicans.

- Oral thrush mostly present as discrete and confluent white plaques on the oral and pharyngeal mucosa, particularly in the mouth and on the tongue.

Characteristic feature of *Candida*

- Candidiasis is *MC systemic* mycosis.
- ***Gram-positive*** fungi characterized by their ability to form *pseudohyphae.*
- Grows rapidly at 25 to 37°C.
- Candida *albicans* (not other species) can also form true hyphae so is dimorphic.
- *Candida albicans* is identified on the basis of their ability to form germ tubes in serum or by formation of thick walled spores called *chlamydospores* on nutritionally deficient media.

10(i). Ans. (c) *Candida* *Ref. DR Arora 2/e, p 671; Clinical Mycology by Elias J Anaessie 2/e, p 2, 11, 631*

Candidiasis is the commonest mycosis involving skin, its appendeges, mucosa and internal organs.

Oral thrush and vulvovaginal candidiasis are commonest manifestation of candida in immunocompetent.

Other important points:

- Aspergillus is the most common fungal sinus infection in immunocompetent patients *...Clinical Mycology by Elias J Anaessie 2/e, p 631*
- Candida is the most common cause of nosocomial fungal infection *...Clinical Mycology by Elias J Anaessie 2/e, P No. 2*
- Cryptococcus neoformans is the most common cause of community associated oppurtinistic fungal infection *....Clinical Mycology by Elias J Anaessie 2/e, P No. 11*
- Cryptococcus neoformans is the most life threatening fungal pathogen.

10 (ii). Ans is (c) i.e Candida Paraspilosis *Ref. Clin Microbiol Rev. Oct 2008; 21(4): 606–625.*

Candida parapsilosis is an emerging major human pathogen that has dramatically increased in significance and prevalence over the past 2 decades, such that C. parapsilosis is now the second leading causes of invasive candidal disease.

- Individuals at the highest risk for severe infection include neonates and patients in intensive care units. *C. parapsilosis* infections are especially associated with hyperalimentation solutions, prosthetic devices, and indwelling catheters, as well as the nosocomial spread of disease ***through the hands of health care workers.***
- Factors involved in disease pathogenesis include the secretion of hydrolytic enzymes, adhesion to prosthetics, and biofilm formation.

Important Points about Candida parapsilosis

- Candida species are presently the ***fourth leading*** cause of nosocomial bloodstream infection.
- ***Candida albicans*** is the ***most frequent*** isolate from blood cultures followed by Candida parapsilosis
- C. parapsilosis ***does not form true hyphae*** and exists in either a yeast phase or a pseudohyphal form.
- C. parapsilosis is notorious for its capacity to grow in total parenteral nutrition and to form biofilms on catheters and other implanted devices, for nosocomial spread by hand carriage.
- C. parapsilosis is of special concern in critically ill neonates, causing more than one-quarter of all invasive fungal infections in low-birth-weight infants. Additionally, it is the predominant fungal organism isolated in many neonatal intensive care units (NICUs), where it is often associated with neonatal mortality
- C. parapsilosis is also a normal human commensal, and it is one of the fungi most frequently isolated from the subungal space of human hands
- C. parapsilosis fungemia can lead to seeding of tissues, resulting in deep-seated infections, and has a mortality rate ranging from 4% to 45%
- Compared to C. albicans, C. parapsilosis more frequently caused fungemia among neonates, in patients with intravenous lines or vascular catheters, who had received prior antifungal agents, were on parenteral nutrition, or had undergone transplantation.

11. Ans. (b) Diagnosis... *Ref. Harrison 19/e, p 1358; 18/e, 1671*

Pneumocystis Jiroveci

- Human isolate of *Pneumocystis* which is associated with severe pneumonia in immunocompromised state particularly AIDS (PcP).
- *P. jiroveci* is an extracellular pathogen. Growth in the lung is limited to surfactant layer above alveolar epithelium.
- Serologic evidence suggest that most individuals are infected in early childhood (thus ***option "c"*** is wrong) but the pneumonia is seen only in immunocompromised state.
- Diagnosis is made by detection of organism in proper specimen.

- **Specimen for *P. carnii* pneumonia (PcP)**
  - *Sputum:* Quick and non invasive.
  - *Broncho-alveolar lavage (BAL) fluid:* ***Mainstay*** of pneumocystis diagnosis.
  - *Transbronchial biopsy:* If diagnosis cannot be made by BAL.
- The overt infection is an ***acute interstitial plasma cell pneumonia*** that occurs with high frequency among two groups:
  - As epidemic of primary infection among premature or debititated or marasmic infant of hospital wards in underdeveloped countries.
  - As sporadic cases among older children and adults who have an abnormal cellular immune status.

12. Ans. (a) and (b) rRNA, mitochondrial protein gene sequence and presence of thymidylate synthase; and cell wall contains glucans *Ref. Harrison 19/e, p 1358, 18/e, p 1671*

- Molecular studies have clearly placed *P. carnii* among the fungi with a close relationship to ascomycetis.
- The classification is based on analysis of gene sequences for ribosomal RNA, mitochondrial proteins, and major enzymes.
- The cell wall of *P. carnii* contain B glucon similar to other fungi.
- In contrast to most fungus *P. carnii* lacks ergosterol and is not susceptible to ergosterol inhibiting antifungal drugs.

Remember: *P. carnii* (now called *P. jeroveci*) is ***MC oppurtunistic infection*** in AIDS patient.

13. Ans. (d) PAS *Ref. Ananthanarayan 8/e, p 601, 9/e, p 592*

"The periodic acid schiff (PAS) and methanamine silver are valuable methods for the demonstration of fungal elements in tissue or biopsy section."

Remember:
- Sabaraud's glucose agar (pH - 5.4), czapek - Dox medium and corn meal agar are most commonly used media in mycology.
- GMS (Gomorris methanamine silver) stain is best fungal stain for biopsy section.

14. Ans. (d) *Aspergillus fumigates* *Ref. Harrison 17/e, p 1256, 1266, 18/e, p 1655*

Aspergillus is a mould with septate hyphae (not have yeast-like morphology).
Another confusing option is ***option "c".***

Penicillum marneffi:

- It is a dimorphic fungi, appearing as small yeast cells in tissue and as a mould in culture.
- It is a leading cause of opportunistic infection in patients of late stage HIV.

Candida is a group of yeast-like fungus except C. albicans which is dimorphic.

Respiratory Infection in AIDS patients		
Bacterial	***Fungal***	***Viral***
S. pneumoniae **(MC)** *H. influenzae* *M. tuberculosis* *Mycobacteria avium complex*	*P.carnii* **(MC)** *Penicillium marneffi* *Aspergillus* Histoplasmosis *Candida* and *Cryptococcus*	CMV

15. Ans. (c) *Trichophyton* *Ref. Chakraborty 2/e, p 611, 622*

Trichophyton is dermatophyton which comes under mould.

Morphological Classification of Fungi	
Yeast	***Yeast-like fungi*** (Grow partly as yeast and partly as chain of elongated budding cells forming pseudohyphae)
– *Cryptococcus neoformans*	– *Candida*
Moulds (Filamentous fungi)- Forms true hyphae	***Dimorphic fungi*** **(Grows either as yeast or as filament)**
– Dermatohyphyte – Zygomycetes – *Malassezia furfur* – Pseudoallescheria species – *Aspergillus* – Penicillium – *Madurella* species – *Philaphora* species	– *Sporothrix schenckii* – *Histoplasma capsulatum* – *Paracoccidiodes brasiliensis* – *Blastomyces dermatitides* – *Coccidiodes immitis* – *Penicilium marneffi* – *Candida albicans* (not other spices of *Candida*)

Mnemonic –SBH Ca Powerful Personal Computer

16. Ans. (b), (c) and (d) *Cryptococcus; Penicillium;* and *Aspergillus* *Ref. Jawetz 27/e, p 674*

Endemic mycosis includes fungal infection which are restricted to specific geographical area.

It includes:
- Histoplasmosis (U.S.A)
- Coccidioidomycosis (U.S.A)
- Blastomycosis *(North American blastomycosis)*
- Paracoccidomycosis *(South American Blastomycosis).*

Fungal Disease in Human		
Superficial Mycoses	***Subcutaneous Mycoses***	***Systemic Mycoses***
– Dermatophytes(includes trichophyton, microsporum, epidermophyton – *Candida* – Ptyriasis/Tinea versicolor – *Tinea nigra*	– Mycotic mycetoma – Chromoblastomycosis – Sporotrichosis – Subcutaneous phycomycosis	– Blastomycosis – Coccidiomycosis – Paracoccidiod mycosis – Histoplasmosis – Oppurtunistic infections (cryptococcus, aspergillus etc.)

17. Ans. (d) *Malassezia furfur* *Ref. Harrison 17/e, p 1263, 18/e, p 1669*

- Malassezia furfur (causative agent of *Tinea versicolor*) were not usually cultured in the clinical laboratory.
- *Cryptococcus, candida, dermatophytes* and *Coccidiodes* are culturable.

Note: Now most epecies can be cultured by adding exogenous lipid in the medium.

Other important feature of Malassezia:
- Part of normal flora of human skin.
- Lipophilic yeast and require lipid in the medium for growth.
- Causative agent of Tinea versicolor and catheter associated sepsis (particularly in infants receiving IV lipid).
- Diagnosis is confirmed by direct microscopic examination of scrappings of infected cells treated with 10-12% KOH with calcoflour white: short unbranced hyphae and spherical cells are observed.
- On inspection with woodlight lesion either do not fluoroscence or appear yellow green.

18. Ans. (a) Fungi imperfecti *Ref. Ananthanarayan 9/e, p 590; Chakraborty 2/e, p 613*

Fungi imperfecti (Deuteromycetes or hypomycetes)
- It is a provisional group of fungi comprising all those in which the sexual or perfect state is not known.
- Most pathogenic fungi belong to this group.

Remember: – *Fungi imperfecti, ascomycetes, basidomycetes produce septate hyphae.*
– *Phycomycetes - fungi with non septate hyphae.*
Ascomycetes – Form sexual spores called ascospores.
Basidomycetes – Form sexual spores called basidiospores

19. Ans. (a) *Cryptococcus neoformans* *See below*

Medical conditions showing maltese cross

Infections:
– Babseia
– Paracoccidiodes
– Cryptococcus
– Malassezia sp.

Lab medicine:
– Urinalysis: 'Maltese crosses' shaped cast are seen in patients of nephrotic syndrome, eclampsia, renal toxicity, fat embolism, after crush injury and in Fabry's disease—due to aggregates of glycosphingolipids

Orthopaedics:
– Arthroscopic fluid following local trauma

Chapter Review

1. **Germ tube test is diagnostic for:** [Kar 04]
   a. *Candida albicans*
   b. *Cryptococcus neoformans*
   c. Histoplasma capsulum
   d. Coccidioidomycosis
   [Ref. Ananthanarayana 8/e, p 607, 9/e, p 612]

> *Candida albicans* forms germ tube when incubated at 37° C in human serum (Reynolds- Braude phenomenon). This property is used for rapid diagnosis of *C. albicans*.

2. ***Pneumocystis carnii* is:** [SGPGI 03]
   a. Bacteria b. Fungus
   c. Virus d. Parasite
   [Ref. Ananthanarayan 9/e, p 614]

3. ***Pneumocystis carinii is* diagnosed by:** [Kerala 01]
   a. Sputum examination for cyst under microscope
   b. Culture
   c. Positive serology
   d. Growth on artificial media
   [Ref. Harrison 19/e, p 1671]

4. **All of the following are yeast-lik fungi except:** [AIIMS 91]
   a. *Candida* b. *Geotrichum*
   c. *Cryptococcus* d. *Trichophyton*
   [Ref. Chakrabirty 2/e, p 611]

5. **Germ tube are seen in:** [RJ 2005]
   a. *Mucor* b. *Candida*
   c. *Aspergillus* d. *Histoplasma*
   [Ref. Ananthanarayan 8/e, p 607, 9/e, p 614]

6. ***Rhinospordium seeberi is a:*** [RJ 2004]
   a. Bacteria b. Algae
   c. Fungi d. Virus
   [Ref. Ananthanarayan 8/e, p 609, 9/e, p 603]

7. ***Pneumocystis carinii* is diagnosed by:** [AIIMS 92]
   a. Silver nitrate staining
   b. Leishmann staining
   c. Fontana staining
   d. Acid fast staining
   [Ref. Harrison 17/e, p 1268, 18/e, p 1671]

8. **Blastomycosis is characterized by all except:** [Delhi 92]
   a. Yeast-like fungus
   b. Commonly involves lung and skin
   c. Dimorphic fungus
   d. Common in South America
   [Ref. Ananthanarayan 8/e, 611, 9/e, p 605]

9. **The following is not true of *Candida albicans*:** [AI 92]
   a. Yeast-like fungus
   b. Forms chlamydospores
   c. Blastomers seen in isolates
   d. Causes meningtis in immunocompromised
   [Ref. Ananthanarayan 8/e, p 607, 9/e, p 611]

10. **Which of the following feature is used for identification of *Cryptococcus neoformans?*** [AP 2009]
    a. Oxidase +ve b. Dextran fermentation
    c. Hydrolyse urea d. Ability to grow at 42°C
    [Ref. Ananthanarayan 8/e, p 610, 9/e, p 613]

> The ability to grow at 37°C and hydrolyse urea differentiates *C. neoformans* from non- pathogenic cryptococci.

11. **The most common type of deep mycosis in India is:** [UP 09]
    a. Blastomycosis b. Histoplasmosis
    c. Coccidioidomycosis d. *Cryptococcus*
    [Ref. Ananthanarayan 8/e, p 610]

12. **Candiasis is frequently associated with all except:**
    a. OCP user b. IUCD user [PGI 93]
    c. Diabetes d. Pregnancy
    [Ref. Harrison 18/e, p 1652]

13. ***Cryptococcus neoformans* is a:** [AI 99]
    a. *Protozoa* b. *Fungus*
    c. *Parasite* d. *Mycoplasma*
    [Ref. Ananthanarayan 8/e, p 610, 9/e, p 613]

14. **True about *Cryptococcus neoformans* is all except:** [AIIMS 96]
    a. Capsular antigen is detected in CSF
    b. Common in immunocompromised patient
    c. Anticapsular antibody prevents recurrence
    d. Strongly positive mucicarmine stain of the organism in tissue is diagnostic

> **Cryptococcal Infection**
> Antibodies are neutralized rapidly by the large amount of capsular antigen released during infection, so capsular antibodies are not protective. Rather cellular immunity play role in protection.

Answers 1. a. *Candida albicans* 2. b. Fungus 3. a. Sputum ... 4. d. *Trichophyton* 5. b. *Candida*
6. c. Fungi 7. a. Silver nitrate ... 8. d. Common in ... 9. c. Blastomers ... 10. c. Hydrolyse...
11. d. *Cryptococcus* 12. b. IUCD user 13. b. Fungus 14. c. Anticapsula ...

15. Which is false regarding *Cryptococcus neoformans*: [AI 95]
a. Grows at 5°C and 37°C
b. It has 4 serotypes
c. Urease negative
d. Causes superficial skin infection
[Ref. Ananthanarayan 8/e p 610, 9/e p 613]

16. *Cryptococcus* is least likely to cause infection of: [AI 95]
a. Skin
b. Bone
c. Brain
d. Kidney

Manifestation of C. neoformans (in decreasing order of frequency).

Common	*Rare*
• Meningoencephalitis ***(MC)***	• Prostatitis
• Pulmonary *cryptococcus*	• Endophthalmitis
• Cutaneous *cryptococcus*	• Hepatitis
• Osteolytic lesion (present as cold abscess)	• Pericarditis
	• Endocarditis
	• Renal abscess

[Ref Harrison 18/e p 1649]

17. Eucalyptus camaldulensis is associated with the transmission of: [PGI 99]
a. Blastomyces dermatitides
b. Histoplasma
c. Cryptococcus
d. *Coccoides immitis* *[Ref. Harrison 17/e p 1252]*

18. Fungi that possess a capsule is: [PGI 99]
a. *Candida*
b. *Aspergillus*
c. *Cryptococcus*
d. *Mucor*
[Ref. Ananthanarayan 8/e p 610, 9/e p 613]

19. Cryptococcus has predilection for: [PGI 98]
a. Lungs
b. Meninges
c. Liver
d. GIT
[Ref. Harrison 17/e p 1252, 18/e p 1649]

20. Which fungal infection is commonest in neutropenia: [AIIMS 99]
a. *Candida*
b. *Histoplasma*
c. *Aspergillus niger*
d. *Aspergillus fumigatus*
[CMDT 14 p 1233]

21. *Candida* infection is predisposed by all, except: [AIIMS 96]
a. Menstruation
b. Diabetes
c. Mini-pill users
d. Combined pill users
[Harrison 18/e p 1652]

22. Cryptococcus capsule is best stained by: [NEET/DNB 07]
a. Methylene blue
b. Crystal violet
c. Indian ink
d. Paraffen *[Ref. Harrsion 18/e 1650]*

23. Candida has predilection for all except: [NEET/DNB 11]
a. Diabetes
b. Extremes of age
c. Athletes
d. Pregnant females
[Ref. Harrison 18/e 1652]

24. P. carnii is primarily a pathogen of: [DNB 12]
a. Rabbit
b. Human
c. Rat
d. Dog
[Ref. Harrison 18/e 1671]

Answers 15. c. Urease... 16. d. Kidney 17. c. *Cryptococcus* 18. c. *Cryptococcus* 19. a. and b
20. a. *Candida* 21. a. Mensturation 22. c. Indian ink 23. c. Athletes 24. c. Rat

NEET Pattern Questions

1. **Virulence factor of cryptococcus includes all, except:**
   a. Polysaccharide capsule
   b. Production of protease
   c. Ability to make melanin
   d. Urease production *[Ref. Harrison, 19/e, p 1340]*

2. **Pneumocystic carnii infects:**
   a. Human
   b. Monkey
   c. Rat
   d. Cats *[Ref. Harrison, 18/e, p 1671]*

P. carnii infects rats; *P. jiroveci* infects human

3. **Renauld Braud phenomenon is seen is:**
   a. Candida albicans
   b. Candida pscitasi
   c. Histoplasma
   d. Cryptococcus *[Ref. AA, 9/e, p 612]*

4. **Which of the following is only yeast:**
   a. Candida
   b. Mucor
   c. Rhizopus
   d. Cryptococcus *[Ref. Harrison, 18/e, p 1665]*

Candida is a yeast-like fungus.

5. **True about cryptococcus are all except:**
   a. Primarily infects lung
   b. Urease negative
   c. India-ink is used
   d. All are true *[Ref. Harrison, 19/e, p 1340]*

Cryptococcus is urease positive fungus.

6. **Most common organism causing fungal infection of oral cavity:**
   a. Candida
   b. Blastomycosis
   c. Aspergillosis
   d. Cryptococcus *[Ref. Harrison, 19/e, p 1342]*

7. **All are true about candida except:**
   a. Pseudohyphae seen
   b. Produce chlamydospore
   c. It is a mould
   d. It is a dimorphic fungus *[Ref. Ananthanarayan, 9/e, p 612]*

Candida produce pseudomycelia, both in tissue and in culture.
Chlamydospore is the thickwalled big resting spore produced by ascomycota (candida); Basidomycota (Panus); It is the life stage which survives in unfavourable conditions.

8. **Stain which differentiates cryptococcus from other fungus:**
   a. Gram Stain
   b. Wright-Giemsa
   c. India ink
   d. Toluidine blue *[Ref. Harrison, 19/e, p 1340]*

9. **Type of pneumonia in P. jiroveci:**
   a. Lobar pneumonia
   b. Interstitial pneumonia
   c. Bronchopneumonia
   d. Any of the above *[Ref. Harrison, 19/e, p 1358]*

Answers 1. d. Urease production 2. c. Rat 3. a. Candida... 4. d. Cryptococcus 5. b. Urease
6. a. Candida 7. c. It is a mould 8. c. India ink 9. b. Interstitial pneumonia

CHAPTER

31 Aspergillus and Mucormycosis

ASPERGILLUS

- Mold with septate hyphae with characteristic dichotomous branching and irregular outline.
- *MC* cause of aspergillosis: *A fumigatus*.
- Aspergillus hyphae are hyaline, narrow and septate with branching at 45°; no yeast forms are present in infected tissue. Hyphae can be seen in cytology or microscopy preparation.
- Out of many species of Aspergillus, only those species that grow at 37°C can cause invasive infection. Although some species without this capability can cause allergic syndromes.

... Harrison 18/e, p 1655, 19/e, p 1345

Types of Aspergillosis

A. Respiratory disease

1. *In healthy person*: Self limited pneumonitis by massive inhalation of spores.
2. *With underlying lung disease*:
   - Allergic bronchopulmonary aspergillosis in patient with asthma, cystic fibrosis: present with wheeze, central bronchiectasis etc.
   - Endobronchial saprophytic pulmonary aspergillosis (Aspergilloma = fungus ball) in cyst or cavity of TB, sarcoidosis, bronchiectasis, histoplasmosis.
   - Often present with hemoptysis. There is no invasion.

B. Superficial infection

- Sinusitis, otomycosis (usually by *A.niger, A. fumigatus*), keratitis etc.
- Otomycosis is *MC* human disease caused by Aspergillus.
- MC radiologic finding is bilateral diffuse or focal pulmonary infiltrates with tendency to cavitate.

C. Disseminated (invasive) Aspergillosis

- ***Lung invasion confined almost entirely to immunosuppressed patients:*** granulocyte count < 500/ml *[MC risk factor: acute leukemia and recipients of tissue transplants]*.
  - Invasion in neutropenic is characterized by hyphal invasion of blood vessels, thrombosis, necrosis and hemorrhagic infarction.
  - **Earliest CT finding**: One or more small pulmonary nodules; *Halo sign* (Hazy rim around infarcted tissue), *Crescent* sign (seen when Bone marrow function recovers) can be seen.
- In HIV patient, *MC* site of aspergillosis: **lung**
- Occur in HIV when **CD_4+<50/µl**, characterized by B/L diffuse or focal infiltrate with a tendency to cavitate.

I

- Mold with septate hyphae: Aspergillus
- Mold with aseptate hyphae: Rhizopus
- MC cause of aspergillosis: A. fumigatus
- MC human disease caused by aspergillus: Otomycosis

I

Primary risk factors for invasive aspergillosis are profound neutropenia and glucocorticoid use

Diagnosis

1. *Microscopy*:
   - Even a single isolate of Aspergillus in KOH mount of sputum of neutropenic patient or hematopoietic stem-cell transplant recipient with pneumonia particularly child or non-smoker suggest diagnosis of invasive disease.
   - PAS stain biopsy of lung, nose, paranasal sinus or sites of dissemination can also be used.
2. *Culture*:
   - Velvety to powdery surface of colony. Ability of *A.fumigatus* to grow at 45°C helps to distinguish it from other species.
   - Culture may be negative or few colonies in aspergilloma or invasive disease.

Treatment

Type of disease	Preferred treatment
Fungus ball	Lobectomy
Allergic bronchopulmonary aspergillosis	Short course of glucocorticoids
Invasive aspergillosis	Voriconazole, Amphotericin B

Multiple Choice Questions

1. **In a patient, corneal scraping reveals narrow angled septate hyphae. Which of the following is the likely etiologic agent:** [AI 02]
   a. *Mucor* b. *Asperagillus*
   c. *Histoplasma* d. *Candida*

2. **Which of the following is the most common etiological agent in paranasal sinus mycoses ?** [AIIMS 06]
   a. *Aspergillus* spp. b. *Histoplasma*
   c. *Conidiobolus coronatus* d. *Candida albicans*

3. **An early diabetic has left sided orbital cellulitis CT scan of paranasal sinus shows evidence of left maxillary sinusitis. Gram stained smear of the orbital exudate shows irregularly branching septate hyphae. The following is most likely etiological agent:** [AIIMS 03]
   a. *Aspergillus* b. *Rhizopus*
   c. *Mucor* d. *Candida*

4. **Branched septate hyphae found on corneal smear in a case of corneal ulcer is:** [AIIMS 00]
   a. *Candida* b. *Mucor*
   c. *Aspergillus* d. *Histoplasma*

5. **Mucor mycosis:** [PGI 02]
   a. Angio-invasion b. Lymph invasion
   c. Septate hyphae
   d. Long term deferoxamine therapy is predisposing factor
   e. It may lead to blindness

6. **Common fungus causing corneal ulcer:** [PGI 01]
   a. *Aspergillus* b. *Mucor*
   c. *Fusarium* d. *Sporthrix*

7. **A young woman complains of recurrent rhinitis, nasal discharge and bilateral nasal blockage since one year. She also had history of allergy and asthma. On examination, multiple polyps with mucosal thickening and impacted secretions are seen in nasal cavities. Biopsy was taken and the material on culture showed many hyphae with dichotomous branching typically at 45 degree. Which of the following is most likely organism responsible?** [AI 2010]
   a. *Rhizopus* b. *Aspergillus*
   c. *Mucor* d. *Candida*

8. **A diabetic patient present with bloody nasal discharge, orbital swelling and pain. Culture of periorbital pus showed branching septate hyphae. Which of the following is the most probable organism involved?** [AI 2010]
   a. *Mucor* b. *Candida*
   c. *Aspergillus* d. *Rhizopus*

9. **Aflatoxin is produced by:** [AI 2011]
   a. *Aspergillus flavus* b. *Candida*
   c. *Aspergillus niger* d. *Penicillum*

Explanations and References with Illustrative Answers

1. **Ans. (b) *Aspergillus*** *Ref. Ananthanarayan 9/e, p 608, 609, 8/e, p 613; Harrison 19/e, p 1345, 18/e, p 1658*
   - *Aspergillus* is mold with septate hyphae with branching at 45°.
   - Asexual conidia are arranged in chain, carried on elongated cells called sterigmata borne on expanded ends of conidiophores.
   - *Aspergillus fumigatus is MC cause of* **aspergillosis.**
   - *Commonest human disease caused by* **aspergillosis** *is otomycosis.*
   - *Aspergillus infection is characterized by hyphae invasion of blood vessel, thrombosis, necrosis, and hemorrhagic infarction.*

   Other options:
   - *Mucor* – Broad nonseptate hyphae in tissue
   - *Histoplasma* – Dimorphic fungi
   - *Candida* – Characterised by presence of pseudohyphae.

   Remember: Aspergillus is MC cause of fungal corneal ulcer.

2. **Ans. (a) *Aspergillus spp.*** *Ref. Dhingra 3/e, p 241*

Many different fungal species are found to involve the paranasal sinuses, the common being *Aspergillus*; Alternaria; Mucor or Rhizopus.

Varieties of fungal infection of sinuses		
•	Fungus ball	Implantation of fungus in a healthy sinus Maxillary sinus is most commonly involved Treatment is surgical removal with no antifungal therapy.
•	Allergic fungal sinusitis	There is no invasion of sinus mucosa by fungus CT shows mucosal thickening with hyperdense area Treatment is endoscopic surgical drainage with pre- and postoperative systemic steroids
•	Chronic invasive	Fungus invades into sinus mucosa CT shows thickened mucosa with opacification of sinus and bone erosion Treatment is endoscopic surgical removal followed by antifungal therapy.
•	Fulminant fungal sinusitis	Acute presentation mostly seen in immunocompromised or diabetic individuals *Most common* fungus associated are mucor and aspergillosis.

3. **Ans. (a) *Aspergillus*** *Ref. Ananthanarayan 8/e, p 613, 9/e, p 609; Harrison 19/e, p 1345 - 1347, 18/e, p 1658*

"Presence of branched septate hyphae in a patient of orbital cellulitis (occur as complication of sinusitis) suggest Aspergillus."

***Aspergillus* Sinusitis occur in three forms:**

1. Ball of hyphae may form in chronically obstructed paranasal sinus, without tissue invasion.
2. A chronic fibrosing granulomatous inflammation begin in sinus and spread slowly to the orbit and brain.
3. Allergic fungal sinusitis

Mucor and Rhizopus belong to family Zygomycetes and have non-septate hyphae.

4. **Ans. (c) *Aspergillus*** *Ref. Ananthanarayan 8/e, p 613, 9/e, p 609; Harrison 19/e, p 1345, 18/e, p 1658*

Already explained

5. **Ans. is (a), (d) and (e) Angio-invasion; Long-term deferoxamine therpay is predisposing factor; and It may lead to blindness** *Ref. Harrison 19/e, p 1346, 18/e, p 1661; CMDT '08; 1336*

Causative agent of Mucormycosis: • Rhizopus • Rhizomucor • Cunninghamella.

They are molds and have non-septate hyphae.

Predisposing factors	
• Recipient of organ transplant	• Hematologic malignancy
• Who are receiving long term desferoxamine therapy	• Diabetics
• Treatment with steroid or cytotoxic drugs	• Chronic renal failure.

Important features of Mucormycosis:

- Vascular invasion by hyphae is a prominent feature.
- Ischemic or hemorrhagic necrosis is foremost histologic findings.

Clinical Manifestations

- *Nose and paranasal sinus infection:*
  - Present with bloody nasal discharge with low grade fever and sinus pain followed in few days by double vision.
  - On examination nasal turbinate of involved side may be dusky red or necrotic.
  - Fungal invasion of ophthalmic artery or orbit may lead to blindness.
  - Coma may occur by direct invasion.
  - Cavernous sinus thrombosis.
- *Pulmonary mucormycosis:*
  - Manifest as progressive severe pneumonia.
  - Hematogenous spread to other areas
- *Cutaneous Mucormycosis:*
  - More common than disease at other site and develop after traumatic injury in which wound are contaminated with dirt.
- *GI invasion:*
  - Present as one or more ulcer which tends to perforate.

Diagnosis

- Microscopic examination and culture of biopsy are essential and swabs are insufficient.
- Organism appears as broad ribbon-like, usually non-septate hyphae with branch at right angles.

Treatment: Wide surgical debridement and intravenous amphotericin B is indicated.

6. **Ans. (a) and (c) *Aspergillus* and *Fusarium*** *Ref. Khurana 2/e, p 123*

Fungi causing corneal ulcer	
• *Aspergillus* (MC)	• Candida
• Fusarium	

7. **Ans. (b) Aspergillus** *Ref. DR Arora 3/e, p 675-677; Harrison 19/e, p 1346, 18/e, p 1658; AA 8/e, p 613, 9/e, p 609*

A fungi with septate hyphae in asthmatic patient can be none other than aspergillus, and this is a case of ***allergic bronchopulmonary aspergillosis***

Allergic bronchopulmonary Aspergillosis

- Seen in atopic individual with elevated IgE levels following sensitization to inhaled aspergillus spores
- In bronchopulmonary aspergillosis, fungus grows within the lumen of bronchioles, which may be occluded. The condition get worsened after development of hypersensitivity to the fungus
- **Diagnosis** is made by an elevated IgE, a positive skin prick test to *A. fumigatus* or detection of *Aspergillus* specific IgE and IgG. Central bronchiectasis is characteristic
- Aspergillus hyphae are hyaline, narrow and septate with branching at 45°; no yeast forms are present in infected tissue. Hyphae can be seen in cytology or microscopy preparation.

Remember: ***Aspergillus can cause:***

- Allergic bronchopulmonary aspergillosis
- Invasive aspergillosis
- Endocarditis
- Paranasal granuloma
- Intracavitary aspergilloma (fungus ball)
- Cutaneous aspergillosis
- Cerebral aspergillosis
- Superficial aspergilloma

8. **Ans. (c) Aspergillus** *Ref. Harrison 19/e, p 1345, 18/e, p 1658-1659*

- Presence of branching septate hyphae in culture suggests the diagnosis of aspergillosis (Mucor and Rhizopus have nonseptate hyphae).
- **Mucor and Aspergillus species** are the most common opportunistic agents causing orbital fungal infections. They usually involve the orbit by direct extension from nasal cavity and paranasal sinuses through bone destruction.

Histomorphical characteristics		
Characteristic	**Aspergillus**	**Zygomycetes (Mucor/Rhizopus)**
• *Diameter*	Narrow (3-6 µm)	Wide (5-20 µm)
• *Caliber*	Uniform	Varying
• *Branching*	Regular, 45° acute angle (dichotomous)	Random, right angle
• *Branching orientation*	Parallel or radial	Random
• *Hyphae*	Usually septate	Usually non-septate

9. **Ans. (a) Aspergillus flavus** *Ref. Ananthanarayan 8/e, p 614, 9/e, p 591, 609*

Aflatoxins are produced primarily by filamentous fungi,Aspergillus flavus and Aspergilus parasiticus.

Mycotic Poisoning	
Mycetism	***Myotoxicosis***
• Eaten Fungus itself produce toxic effects • Cause GI disease, dermatitis • Hallucinogenic agents (d-lysergic acid, Psilocybin) produced by psilocybe species have been used for medicinal purpose	• Fungal toxins contaminate some articles of food examples include: – Aflatoxin produced by Aspergillus flavus – Ergotoxicosis produced by claviceps purpura

Aflatoxin: MC Mycotoxicosis

- It is frequently found in moudly foods, like ground nut, corn, peas
- It is highly toxic to animals, birds and human beings
- It has got carcinogenic effect in liver.

NEET Pattern Questions

1. Which of the following is an aseptate fungus [NEET/DNB 2012]

a. Aspergillus
b. Candida
c. Nocardia
d. Rhizopus

[Ref. Greenwood 18/e 639]

2. Bronchopulmonary aspergillosis is mediated by:

a. Type I hypersensitivity
b. Type III hypersensitivity
c. Type II hypersensitivity
d. Both a and b

[Ref.Ananthanarayan 9/e 609]

ABPA is mediated either by type I hypersensitivity or type III hypersensitivity (extrinsic alveolitis) or combined type I and type III hypersensitivity

3. Aseptate hyphae is not seen in:

a. Rhizopus
b. Mucor
c. Aspergillus
d. None *[Ref. Harrison 18/e 1658, 19/e, p 1345]*

Aspergillus hyphae are hyaline, narrow and septate with branching at 45°; no yeast forms are present in infected tissue.

4. Aseptate hyphae are seen in:

a. Phycomycetes
b. Ascomycetes
c. Basidiomycetes
d. Deutromycetes *[Ref. Ananthanarayan 9/e 1658]*

Phycomycetes are lower fungi that have non septate hyphae and form endogenous asexual spores called sporangiospores.

Answers 1. d. Rhizopus 2. d. Both a and b 3. c. Aspergillus 4. a. Phycomycetes

CHAPTER

32

Dimorphic Fungi

- **Dimorphic fungus occur in 2 forms:**
  1. **Yeast form = parasitic phase**
     In host tissues and on cultures at 37°C (enriched agar).
  2. **Spores and filamentous (mould) form = saprophytic phase**
     In soil and culture at 22-25°C or Sabourauds agar at room temperature.
- Disease by all of them are restricted to specific areas of endemicity.
- **Dimorphic fungus are:** *Candida albicans, Histoplasma, Sporothrix schenckii,* Blastomycosis, Coccidioidomycosis, Paracoccidiodomycosis, Penicillium marneffi.

Mnemonic = ***Senior Boys Hostel Ca Powerful Personal Computer.***
= ***His Pen Can Blast Spores of Coccido and Paracoccido***

HISTOPLASMA CAPSULATUM

- *Non-capsulated* intracellular (in macrophages) fungus with septate hyphae. Cause primarily a disease of reticuloendothelial system.

I
Histoplasma
- Non-capsulated, intracellular fungus with septate hyphae
- Source of infection: inhalation of spores
- Clinically histoplasmosis mimics TB

Source of infection

- Inhalation of spores present in moist surface of alkaline soil enriched by dropping of birds and bats.

Pathology

It forms 2 types of asexual spores, large tuberculate macroconidia and smaller elliptical microconidia. Microconidia reach the alveoli and initiate granulomatous reaction.

I
Histoplasma is also called Darling disease or cave's disease

Clinical features

1. Majority of infections are asymptomatic or mild *(acute primary pulmonary histoplasmosis).*
   - Cough, fever, malaise, and chest X-ray finding of hilar adenopathy due to caseation necrosis or calcification (which mimics TB) with or without 1 or more areas of pneumonitis are typical features.
2. Small proportion of patient *(who lack history of acute primary pulmonary disease)* develop progressive disease in either form:
   - Chronic pulmonary histoplasmosis or chronic fibrocavitary pneumonia.
   - Disseminated disease: Among immunosuppressed and user of TNF α antagonist infliximab. It mimics disseminated TB.

I
Tuberculate spores are characteristic of Histoplasma

Diagnosis

Culture (Preferred method):

- *Tuberculate spore is diagnostic*
- Sputum culture - For chronic pulmonary histoplasmosis.
- Culture of bone marrow, mucosal lesion, liver and BAL fluid are diagnostically useful in disseminated histoplasmosis.
- Blood culture is best performed by lysis centrifugation method.

Treatment

Disease	Preferred treatment	Alternative
Acute pulmonary	None	
Chronic pulmonary	Itraconazole	Amphotericin B
Disseminated Severe illness • CNS involvement • Immunocompromised	Itraconazole Amphotericin B	Amphotericin B

BLASTOMYCOSIS (=NORTH AMERICAN BLASTOMYCOSIS)

B. dermatitidis is Dimorphic fungi with septate hyphae forming *conidiophores bearing single globose* to piriform conidia. Most cases are found in North America.

Source of infection: Inhalation of conidia from warm moist soil of wooden areas.

Clinical features

It has marked predilection for lungs, skin and bone.

- Majority of patient, have chronically progressive course and minority have self limited pneumonia.
- Cutaneous disease is usually on the face or other exposed parts of the body in the form of elevated ulcerative lesions.

Diagnosis:

Demonstration of fungus in culture of sputum, pus or urine.

Treatment:

- Rapid progression or severe illness → Amphotericin B
- CNS disease → Amphotericin B
- Mild to moderate and no CNS disease → Itraconazole.

COCCIDIOIDOMYCOSIS

- C. immitis is Dimorphic fungi with septate hyphae forming barrel shaped (arthospores) or arthroconidia and non-budding spherules with endospores (tissue form).
- It is present in soil and rodents.
- Infection is acquired by inhalation of dust containing arthospore.
- Majority of person develop asymptomatic respiratory infection.
- Some develop self limited influenza like fever known as *valley fever* or *desert rheumatism.*
- Very few develops coccidiodal granuloma often with caseation necrosis.

Diagnosis

a. Sputum, urine and pus should be examined by wet smear and culture (arthrospores are formed in chain from alternate cells of septate hyphae).
b. On *biopsy* appearance of *mature spherule is diagnostic.*
c. Serological test are very helpful.

I

Blastomycosis
- Dimorphic fungi with septate hyphae shows predilection for lungs, skin and bones

Coccidioidomycosis
- Dimorphic fungi with septate hyphae
- Clinically present with influenzae like illness

PARACOCCIDIODES BRASILLIENSIS

- Dimorphic fungi with septate hyphae + rare globose conidia and chlamydospores.
- Tissue form: Yeast with characteristic multiple budding.
- Causative agent of *'South American Blastomycosis'* in which *pulmonary infection spreads hematogenously to* mucosa of mouth; nose; lymph node; skin and other internal organs (e.g. adrenal, GIT).
- Ulcerative granuloma of buccal and nasal mucosa are prominent feature of disease.

Diagnosis

Culture of sputum, pus and mucosal lesion are often diagnostic.

Treatment

- Mild disease - Itraconazole
- Advanced disease - Amphotericin B

SPOROTHRIX

Sporothrix schenckii is thermally ***dimorphic fungi*** causing subcutaneous mycoses (**Note:** Other dimorphic fungi cause systemic mycosis).

Colonies are blackish (variation in pigmentation) and shiny but becomes wrinkled and fuzzy with age.

Source of Infection

Acquired from inoculation via thorn pricks of rose, sphagnum moss etc. into subcutaneous tissue through minor trauma.

I

Sporothrix

Dimorphic fungi causing subcutaneous mycosis in the form of multiple ulcers

Clinical feature

- Most cases occur in upper limb.
- Usual site of infection: extremity (facial lesion in case of children).
- Sporotrichosis is characterized by development of nodules on skin, subcutaneous tissue and in lymph nodes, which soften and break to form indolent ulcers.
- It is of following types:
  a. ***Plaque sporotrichosis***: Non-tender lesion confined to site of inoculation.
  b. ***Lymphangiitis sporotrichosis***: It is *MC* manifestation in which secondary lesion are seen along the lymphatic channels. These small painless nodules may ulcerate and exudate pus.
  c. **Extracutaneous sporotrichosis**: Portal is probably lung. Pulmonary sporotrichosis is usually present as single chronic cavitary upper lobe lesion.

Diagnosis

- *Culture* (most reliable) of pus, joint fluid, sputum or skin biopsy in which septate hyphae carrying flower like cluster of small conidia is seen.
- In tissue – Fungus is seen as 'cigar shaped yeast' yeast cell without mycelia.
- Sometimes *'Asteroid Bodies'* can be seen which is formed due to antigen-antibody reaction.

Treatment

- Cutaneous sporotrichosis – *DOC* Itraconazole
- Alternative potassium iodide
- Extracutaneous sporotrichosis – IV Amphotericin B is drug of choice
- Alternative Itraconazole.

Multiple Choice Questions

1. **A series of ulcers in lower extremities in sub-Himalayan area is often caused by:** [AI 2012, 97]
   a. *Trichophyton rubrum* b. *Pseudallescheria boydii*
   c. *Cladosporium species* d. *Sporothrix schenckii*

2. **What is true about Histoplasmosis:** [AIIMS 08]
   a. In early stages it is indistinguishable from TB
   b. Blood culture is not diagnostic
   c. Hyphal forms are infectious form
   d. Person to person spread occurs by droplet infection

3. **A patient resident of Himachal Pradesh presented with a series of ulcers in a row, on his right leg. The biopsy from affected area is taken and cultured on sabauraud's dextrose agar. What would be the most likely etiological agent?** [AIIMS 03]
   a. *Sporothrix schenckii*
   b. *Cladosporium* sp
   c. *Pseudo allescheria boydii*
   d. *Nocardia brasiliensis*

4. **The following fungi are thermally dimorphic, except:**
   a. *Sporothrix schenkii* [AIIMS 03]
   b. *Cryptococcus neoformans*
   c. *Blastomyces dermatitidis*
   d. *Histoplasma capsulatum*

5. **Dimorphic fungus are:** [PGI 02]
   a. *Candida* b. *Cryptococcus*
   c. Blastomycosis d. Coccidiomycosis
   e. Sporotrichosis

6. **All are dimorphic, except:** [AIIMS May 09]
   a. *Blastomyces* b. *Histoplasma*
   c. *Penicillium marneffei* d. *Phialophora*

7. **A gardener has multiple vesicles on hand and multiple eruptions along the lymphatic. Most common fungus responsible is:** [AIIMS 08]
   a. *Sporothrix schenckii* b. *Clasdosporium*
   c. *Histoplasma* d. *Candida*

8. **A farmer from the sub-himalayan region presents with multiple leg ulcers. The most likely causative agent is** [AIIMS Nov 2012]
   a. Trichopyton rubrum b. Cladosporium species
   c. Sporothrix schenkii d. Aspergillus

Explanations and References with Illustrative Answers

1. **Ans. (d) Sporothrix schenckii** *Ref. Harrison 19/e, 1353 - 1354; 18/e p 1685*

Important Features of Sporothrix

- Thermally dimorphic fungus lives as saprophyte on plants.
- Infection results from inoculation into subcutaneous tissue after minor trauma.
- Nursery worker, florist, gardeners acquire the illness from roses, and other plants.
- Usual manifestation is nearly painless red papule at the site of inoculation, over the next several weeks similar nodules forms along proximal lymphatic channels. The nodules may ulcerate. Thus series of ulcer may form.

Diagnosis :
- In skin lesion the organism is hard to find.
- Culture of pus or a skin biopsy is preferred method of diagnosis.

Treatment:
- Potassium iodide
- Itraconazole.

2. **Ans. (a) In early stages it is indistinguishable from TB** *Ref. Harrison 19/e, p 1353, 18/e, p 1641*

Clinical manifestation of Histoplasma

1. Majority of infections are asymptomatic or mild (acute primary pulmonary histoplasmosis).
   - Cough, fever, malaise. Chest X-ray shows hilar adenopathy due to caseation necrosis or calcification (which mimics TB) with or without 1 or more areas of pneumonitis.
2. Small proportion of patient (who lack history of acute primary pulmonary disease) develop progressive disease in either form:
   - Chronic pulmonary histoplasmosis or chronic fibrocavitary pneumonia.
   - Disseminated disease: Among immunosuppressed and user of TNF α antagonist infliximab. It mimics disseminated TB.

Other Options:

- Fungal culture is the gold standard diagnostic test for histoplasmosis.
- Mycelia are the naturally infectious forms.
- Infection occurs through aerosolization of bird or at dropping.

3. **Ans. (a)** ***Sporothrix schenckii*** *Ref. Harrison 19/e, p 1354; 18/e, p 1685*

Already explained

4. **Ans. (b)** ***Cryptococcus neoformans*** *Ref. Chakraborty 2/e, p 611*

Don't get confuse with term thermally dimorphic as it is same as dimorphic fungi i.e. fungi that occur as yeast at body temperature and mould in soil at 22-25°C.

5. **Ans. (c), (d) and (e) Blastomycosis, Coccidioidomycosis and Sporotrichosis** *Ref. Chakraborty 2/e, p 611; Jawetz 25/e, p 647*

Dimorphic fungus

- Fungus which occur in two forms:
  - ***Yeast form*** - In host tissue and on culture at 37°C.
  - ***Filamentous (mold) form*** - In soil and culture at 22 - 25°C or Sabouraud's agar at room temperature.

Dimorphic fungus are:

- *Sporothrix schenckii*
- *Blastomyces*
- Coccidioidomycosis
- *Candida albicans* (not other species of candida)
- *Penicillium marneffii*
- *Histoplasma*
- Paracoccidioidomycosis

Mnemonic: **SBH Ca Powerful Personal Computer**

Remember: Candida as a whole is not dimorphic only *Candida albicans* is dimorphic.

6. **Ans. (d)** ***Phialophoro*** *Ref. Jawetz 24/e, p 645, Ananthanarayan 8/e, p 601, 9/e, p 590*

Already explained

Remember: Phialophora is a dermatiaceous (pigmented fungi) soil fungi which can cause subcutaneous or intramuscular lesion with abscess or cysts containing masses of brown hyphae.

7. **Ans. (a)** ***Sporothrix schenckii*** *Ref. Harrison 19/e, p 1353, 18/e, p 1685*

Already explained

8. **Ans. is (c) sporothrix *schenckii*** *Ref. Harrison 19/e 1353*

Already explained

Chapter Review

1. **Which among are dimorphic fungi:** [Jharkhand 05]
   a. Blastomycosis b. Paracoccidioidomycosis
   c. Histoplasmosis d. Otomycosis
   [Ref. Ananthanarayan 8/e, p 601, 9/e, p 590]

2. **All of the following regarding histoplasmosis are true, except:** [MP 06]
   a. Very rare in patients with AIDS
   b. Bone marrow is involved
   c. Gomori methamine silver stain used
   d. Dimorphic fungus
   [Ref. Harrison 17/e, p 1245; Robbins 7/e, p 754 - 755]

3. **All of the following are dimorphic fungi, except:** [AI 97]
   a. *Sporotricum* b. *Blastomycetes*
   c. *Histoplasma* d. *Cryptococcus*
   [Ref. Jawetz 25/e p 647]

4. **All are examples of dimorphic fungi, except:**
   a. *Histoplasma capsulatum* [AIIMS 97]
   b. *Blastomyces dermatitidis*
   c. *Cryptococcus neoformans*
   d. *Coccidiodies immitis* *[Ref. Jawetz 25/e p 647]*

5. **Asteroid bodies and cigar shaped globi may be produced by:** [DNB 2013]
   a. Sporothrix
   b. Sporotrichosis
   c. Phialophora
   d. Aspergillus
   [Ref. Ananthanarayan 9/e, p 602]

6. **Histoplasmosis is spread by:** [DNB 2013]
   a. Human b. Water
   c. Soil d. None
   [Ref. Ananthanarayan 9/e p 607]

Answers 1. a. Blastomycosis 2. a. Very rare in patients ... 3. d. Cryptococcus 4. c. Cryptococcus ...
5. a. Sporothrix 6. c. Soil

NEET Pattern Questions

1. **Dimorphic fungi behaves like yeast at:**
   a. < 10°C b. Body temperature
   c. > 40°C d. In vitro
   [Ref. Ananthanarayan, 9/e 590]

Dimorphic fungi grows as yeast in culture at 37°C and in tissues

2. **Darling disease is caused by:**
   a. Histoplasma b. Candida
   c. Cryptococcus d. Rhizopus
   [Ref. Ananthanarayan, 9/e 607]

Histoplasmosis is also known is darling disease, or Caves disease or caver's disease.

3. **Dimorphic fungus:**
   a. Candida b. Histoplasma
   c. Rhizopus d. Mucor
   [Ref. Ananthanarayan, 9/e 590]

4. **Which of the following fungi is/are difficult to isolate culture:**
   a. Candida b. Dermatophytes
   c. Cryptococcus d. Malassezia furfur
   [Ref. Ananthanarayan, 9/e 590]

5. **Not true about Histoplasma capsulatum:**
   a. Dimorphic fungus b. May mimic TB
   c. Capsulated d. Mostly asymptomatic
   [Ref. Harrison, 19/e 1332]

6. **Asteroid bodies are seen in:**
   a. Cryptococcosis b. Histoplasmosis
   c. Sporotrichosis d. Aspergillosis
   [Ref. Ananthanarayan, 9/e 602]

Asteroid bodies are the characteristic feature of sporotrichosis. They are seen on histological examination of tissue sections stained by methenamine silver stain. They are rounded or oval, basophilic, yeast-like body 3-5 μm in diameter.

7. **"Tuberculate spores" are characteristic features of:**
   a. Candida b. Histoplasma
   c. Coccidiodomyces d. Cryptococcus
   [Ref. Ananthanarayan, 9/e 608]

Answers 1. b. Body temperature 2. a. Histoplasma 3. b. Histoplasma 4. d. Malassezia furfur
5. c. Capsulated 6. c. Sporotrichosis 7. b. Histoplasma

Section - B

UNIT – IV Parasitology

- Basics of Parasitology
- Protozoa
- Helminths

CHAPTER

33

Basics of Parasitology

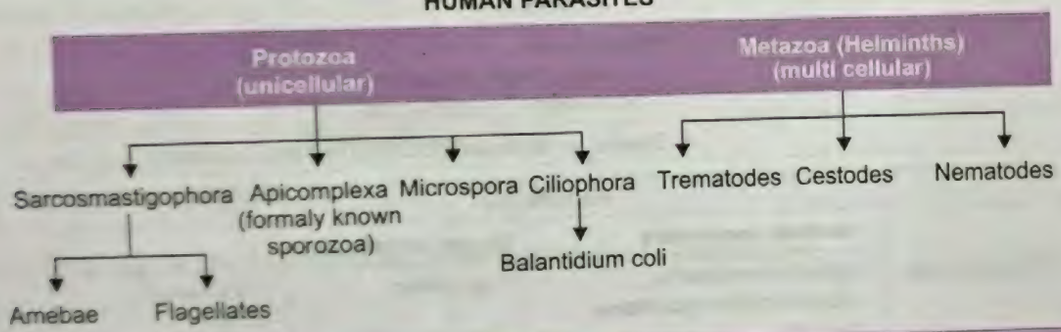
PARASITE

Ectoparasites: Parasite inhabiting the body surface only, e.g. lice, ticks, mites. The term infestation is used in context of ectoparasites.
Endoparasites: Parasite, living inside the body.

HOST

a. **Definitive host:** Host in which adult stage lives or the **sexual** mode of reproduction takes place.
b. **Intermediate host:** Species in which the larval stage of parasite lives or **asexual** reproduction takes place.
c. **Paratenic host:** A host in which parasite remains viable without development or multiplication. Such host is also called as transport host.

HUMAN PARASITES



AMEBAE	
Amebae of alimentary canal	**Pathogenic free living amebae (Brain parasite)**
• *Entameba histolytica*	• *Naegleria fowleri*
• *Entameba coli*	• Acanthameba
	• Balamuthia

Flagellates	
Intestinal flagellates	**Hemoflagellates (Kinetoplastidia)**
• Giardia lamblia	• Trypanosoma
• Trichomonas	• Leishmania

Sporozoa (Apicomplexa)		
• **Apicomplexa:** Members possess a structure called as apical complex, at some stage of their life cycle. Apical complex serve as organ of attachment. They are tissue parasites.		
• *Plasmodium*	• *Toxoplasma gondii*	• *Sarcocystis*
• *Isospora belli*	• *Cryptosporidium parvum*	• *Babesia*
• *Cyclospora*		

TREMATODES (FLUKES)	
Dioecious blood flukes (Schistosomes) [infection by cercarial penetration]	***Hermaphrodites flukes [infection by ingestion of cercaria]***
• *S. hematobium*/bilharziasis hematobium [live in vesical and pelvic venous plexus]	*Biliary tract (liver flukes)* – *Clonorchis sinensis* – *Fasciola hepatica* – *Opisthorchis species*
• S. mansoni/ Intestinal bilharziasis [live in inferior mesenteric vein]	Intestinal flukes – Small intestine → *Fasciolopsis, buski*, etc – Large intestine → Gastrodiscoides hominis
• *S. japonicum*/oriental schistosomiasis/ Katyama disease [live in superior mesentric vein]	• Lung flukes (*Paragominus westermani*)

CESTODES (TAPEWORMS)	
Operculated eggs, ciliated larvae	***Non-operculated eggs Non-ciliated larvae (bladder worms)***
• *Fish tapeworm (Diphyllobothrium latum)*	• *Taenia* *T.* – *saginata* (Beef tapeworm) *T.* – *solium* (Pork taperworm)
• *Sparganum*	• Echinococcus – *E. granulosus* (Dog tapeworm) – E. multilocularis
	• Hymenolepsis – *H. nana* (dwarf tapeworm) – *H. diminuta* (rat tapeworm)
	• *Dipylidium caninum* (double pored dog tapeworm)

Nematodes			
	Intestinal nematodes	**Tissue nematodes**	
• *Small intestine*	– Ascaris/roundworm – Hookworm/Ancyclostoma – Necator – Strongyloides – Trichinella	• Lymphatic	– Wuchereria – Brugia
		• Subcutaneous	– Loa-loa – Onchocerca – Dracunculus (Guinea worm/serpent worm)
• *Large intestine*	– Enterobius (Pinworm/ threadworm/ seatworm) – Trichuris (Whipworm)	• *Mesentery*	– Mansonelia
		• Conjunctiva	– Loa-loa

Nematodes can also be Classified as

- **On the basis of mode of infection**
  1. *By ingestion*
     a. Eggs - *Enterobius, Ascaris, Trichuris* **Mnemonic: EAT**
     b. Larvae within intermediate host – *Dracunculus*
     c. Encysted larvae in muscle – *Trichinella.*
  2. *By penetration of skin - Ancylostoma, Necator, Strongyloides* **Mnemonic: ANS**
  3. *By blood sucking insects* – Filariae
  4. *By inhalation of Dust Containing eggs - Ascaris, Enterobius.*
- **Based on whether they lay eggs or larvae:**
  1. *Oviparous = Laying eggs*
     a Unsegmented eggs : *Ascaris, Trichuris*
     b. Segmented eggs : *Ancylostoma, Necator* **Mnemonic: ANS**
     c. Eggs containing larvae : *Enterobius*

2. *Viviparous = Producing larvae*
   - Trichinella - Wuchereria
   - Brugia - Dracunculus.
3. Ovoviviparous (laying eggs containing fully formed larvae which hatch out immediately)
   - Strongyloides

Two Intermediate hosts are seen in:

- *Paragonimus westermani* (Lung fluke)
- *Diphyllobothrium latum* (Fish tape worm)
- *Clonorchis sinesis* (Chinese tapeworm)
- *Metagonimus yokogawai.*

Man is intermediate (Secondary) host in:

- *Plasmodium*
- *Toxoplasma gondii*
- *Sarcocystis lindemanni*
- *T. solium* (man also act as definitive host).
- *Echinococcus granulosus* [*dog tapeworm/ hydatid worm/Taenia echinococcus*]

Note: In other parasitic infection, man act as definitive (primary) host.

Auto-Infection occurs in:

- Cryptosporidium parvum
- *H. nana*
- *E. vermicularis*
- *T. solium*
- *Strongyloides stercoralis.*

Mnemonic: CHEST

Eggs float (eggs can be demonstrated) in concentrated saturated solution:

- *E. granulosus*
- *H. nana*
- All nematodes (but not unfertilized egg of *Ascaris*).

Charcot Leyden crystal seen in:

- *E. histolytica* (amoebic dysentery)
- Whipworm dysentery
- *Ascaris pneumonia*
- Bronchial asthma.

Worms that crawl out:

- *Enterobius vermicularis*
- *T. saginata.*

Worms which do not multiply in host:

- *Ancylostoma duodenale*
- *Enterobius vermicularis*
- *W. bancrofti.*

Parasites associated with malignancy:

- Clonorchis
- Opisthorchis

] Bile duct carcinoma

- Schistosoma hematobium - bladder carcinoma.

Premunition (immunity to reinfection) seen in:

- *Syphilis*
- *Cutaneous leishmaniasis.*
- Hyper/Holoendemic malarial area

Cystic stage is absent in:

- *Dientamoeba fragilis*
- *Entamoeba gingivalis*
- *Trichomonas vaginalis*
- *Trichomonas intestinalis.*

Undulating membrane seen in:

- Trichomonas species
- Hemoflagellates

IMPORTANT FACTS

Only ***'Protozoan'*** parasite found in lumen of human small intestine — *Giardia lamblia*

Only ***'Ciliate protozoan'*** Parasite of man—*Balantidium coli*

Parthenogenic worm (female is able to produce fertile eggs or larvae without meeting with males): *Strongyloides stercoralis*

Largest protozoa: *Balantidum coli*

Smallest intestinal amoeba: *Dientamoeba fragilis*

Smallest and most common tapeworm found in human intestine: *H. nana*

Largest helminth *T. saginata* (beef tapeworm)

Largest liver fluke *F. hepatica*

Largest trematode infecting man - *Fasciolopsis buski*

Largest nematode *Ascaris* (roundworm)

Smallest nematode *Trichinella*

MC protozoan parasite *Toxoplasma gondii*

Dogs are responsible for transmission of:

- Hydatid disease
- *Toxocara canis*
- *L. donovani infantum*

Eggs needs development in soil:

- *Ancylostoma duodenale*
- *Ascaris*
- *Trichuris (whipworm)*
- *S. stercoralis.*

Sputum examination done for:

- Rhabditiform larva of *Ascaris*
- Golden brown - eggs of paragonimus
- Filariform larva of *Strongyloides, Ancylostoma*
- *Entamoeba histolytica* (due to hepatobronchial fistula).
- *Paragoninus westermani*

Cutaneous larva Migrans caused by:

- *Necator americanus*

- Sparganosis
- *Gnathostomiasis*
- Hypoderma and gastrophilus.
- Loa-loa and *Dicrofilaria*
- *Ancylostoma braziliense, A. caninum, A. duodenale*
- *Fasciola* and *Paragonimus*
- *Strongyloides stercoralis* (larva currens/racing larvae).

Visceral Larva Migrans caused by:

- Dog ascarid *Toxocara canis (MC)*
- Cat ascarid *T. cati*
- Anisakis (Large ascarid)
- *Gnathostoma.*

Worms Pass through lung during its life cycle:

- *Schisto. hematobium* (Lung act as 2nd filter)
- *Paragonimus*
- *Echinococcus*
- *Strong. sterocoralis*
- *A. dudodenale*
- *Ascaris.*

Intracellular Parasites:

- *Leishmania* (amastigote form)
- *Babesia*
- *Plasmodium*
- *Toxoplasma gondii*
- *Sarcocystis.*
- *Trypanosoma cruzi* (amastigote form)

NEUROPARASITES

Protozoa	Helminthes		
	Larvae of cestodes	Nematodes	Ectopic ova
• *E. histolytica*	• *T. solium*	• Visceral larva migrans	• *Schistosoma sp (hematobium)*
• *Naegleria*	• *E. granulosus*	• *Ascaris lumbricoides*	• *F. hepatica*
• *Acanthamoeba*	• *Multiceps sp.*	• *Strongyloides stercoralis*	• *Heterophyes hyterophyes*
• *Trypanosoma*		• *Gnathostoma spinigerum*	
• *P. falciparum*			
• *T. gondii*			

Protozian parasites causing diarrhea	
• Giardia lamblia	• Entamoeba histolytica
• Cyclospora cayetanensis	• Cryptosporidim parvum
• Isospora belli	

Protozoa parasites detected in peripheral blood film

- Trypanosoma cruzi
- Tryanosoma brucei rhodesiense
- Trypanosoma brucei gambiense
- Leishmania spp.
- Plasmodium spp.
- Babesia spp.

Protozoa transmitted by sexual contact

- Trichomonas vaginalis
- Giardia lamblia
- Entamoeba histolytica

Parasites which can be transmitted from mother to fetus

- Toxoplasma gondii
- Plasmodium spp.
- Trypanosoms cruzi

Acid fast parasitic organisms

- Microsporidia (spore)
- Cyclopora cayetanensis (oocyst)
- Isospora belli (oocyst)
- Cryptosporidium parvum (oocyst)

Parasites causing opportunistic infections in immunocompromised patients (HIV-positive cases

- Microporidia
- Cyclospora cayetanensis
- Isospora belli
- Cryptosporidium parvum
- Toxoplasma gondii
- Strongyloides stercoralis
- Entamoeba histolytica

Parasites which can be cultured in laboratory	
• Balantidium coli	• Entamoeba histolytica
• Acanthamoeba spp.	• Giardia lamblia
• Trichomonas vaginalis	• Trypanosoma spp.
• Leishmania spp.	

- Longest cestode infecting man: Diphyllobothrium latum
- Smallest cestode infecting man: Hymenolepis nana
- Largest trematode infecting humans: *Fasciolopsis buski*
- Smallest trematode infecting humans: *Heterophyes*

Parasites with aquatic vegetations as the source of infection	
• Fasciola hepatica	• Fasciolopsis buski
• Gastrodiscoides hominis	• Watsonius watsoni

Parasites with bile stained eggs	
• Ascaris lumbricoides	• Clonorchis sinensis
• Trichuris trichiura	• Fasciola hepatica
• Taenia solium	• Fasciolopsis buski
• Taenia saginata	

Parasites found in urine

- Wuchereia bancrofti
- Schistosoma hematobium
- Trichomonas vaginalis

Parasites found in cerebrospinal fluid	
Protozoa	**Helminths**
• Trypanosoma brucei spp.	• Angiostrongylus cantonensis
• Naegleria	
• Acanthamoeba spp.	

Parasites found in peripheral blood film	
Protozoa	**Nematodes**
• Plasmodium spp.	• Wuchereia bancrofti
• Babesis spp.	• Brugia spp.
• Leishmania spp.	• Loa loa
• Tryponosoma spp.	• Mansonella ozzardi

Parasites with mosquito as intermediate host
• Wuchereria bancrofti
• Brugia spp.
• Mansonella spp.
• Dirofilaria spp.

Helminths requiring no intermediate host
• Ancylostoma duodenale
• Necator americanus
• Ascaris lumbricoides
• Trichuris trichiura
• Enterobius vermicularis
• Hymenolepis nana

Small Intestinal Parasites		
Protozoa	**Nematodes**	**Cestodes**
• Giardia lamblia	• Stronglyoides stercoralis	• Diphyllobothrium latum
• Isospora belli	• Ascaris lumbricoides	• Taenia solium
• Cyclospora caytenensis	• Ancylostoma duodenale	• Taenia saginata saginata
• Sarcocystis hominis and suihominis	• Necator americanus	• Taenia saginata asiatica
	• Trichinella spiralis	• Hymenolepis nana
	• Trichostrongylus spp.	
	• Capillaria philippinensis	

IMPORTANT POINTS ABOUT MALARIA

- Infective forms for human is sporozoites in saliva of mosquito.
- Infective forms for mosquito is gametocytes in human blood. At least 12 gametocytes per cubic mm of blood must be present to infect mosquito.
  - Gametocytes are maximum in number during the early stages of infections (may exceed 1000 per cubic mm of blood).
  - Nonmotile zygote converted into motile ookinete in about 18-24 hours.
- Human reservoir is one who harbors the sexual forms (gametocytes) of the parasite.
- Only animal reservoir is chimpanzees.

CHAPTER

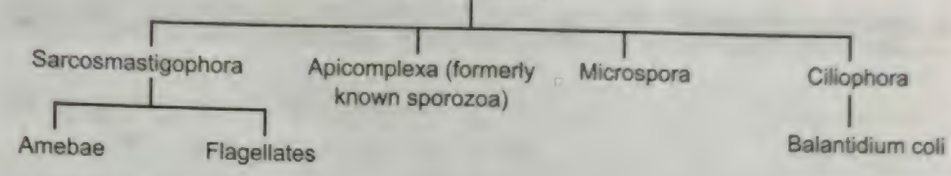
34

Protozoa

I
- Phylum microspora contains intracellular protozoan parasites which affect immunocompromised individuals only.

- Single-celled eukaryotic microorganisms belonging to kingdom protista.
- Represent earliest form of animal life.

Classification



I
Largest protozoan Parasite: Balantidium coli

IMPORTANT PROTOZOANS

ENTAMOEBA HISTOLYTICA

- Found in human colon, as commensal, but sometimes invades the intestinal tissue and becomes pathogen.

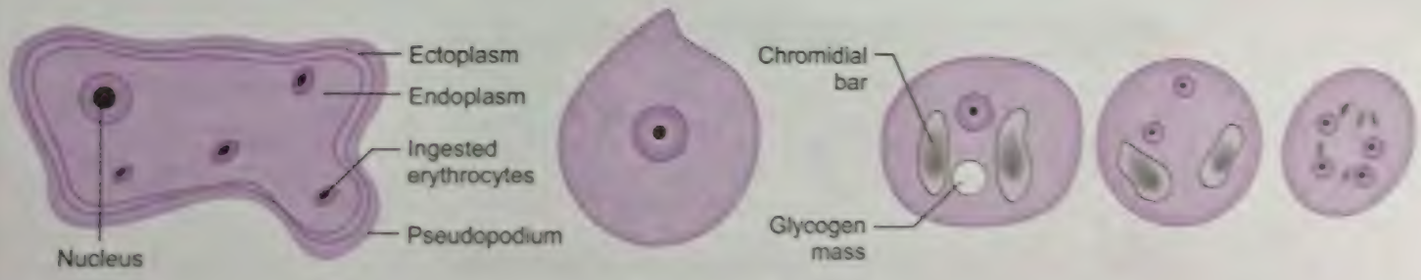
E. – **It has 3 stages:**

a. **Trophozoite or Vegetative** or Amoeboid form
   - It is the only form present in tissue.
   - It can't initiate infection.

b. **Pre-cystic stage**
   - Encystment occur in intestinal lumen only (not in feces or nor in tissues).
   - Contain no RBC and other ingested food particles.

c. **Cystic stage**
   - Mature Quadrinucleate cyst: *Infective stage*
   - It is present *only in lumen* of colon and in mushy or formed feces.
   - When stained with iodine, nuclear chromatin and karyosome appears bright yellow while chromidial bars are unstained.

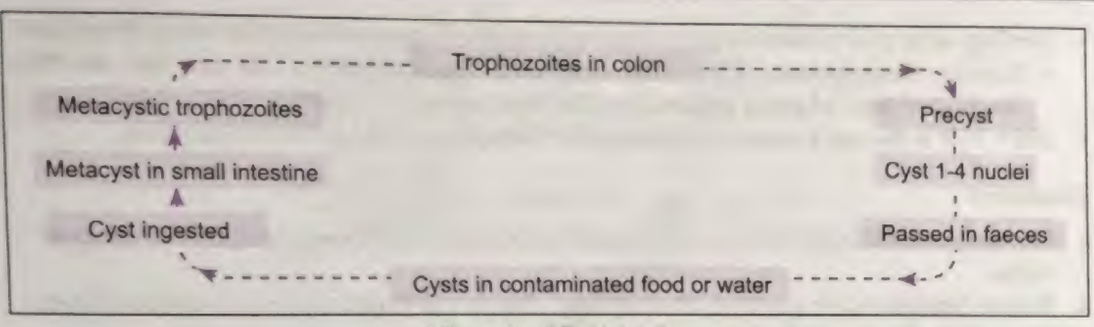
I
E. histolytica
- Infective stage: cyst
- Tissue stage: trophozoite



Life Cycle

I
Fecoral is the commonest mode of transmission. Beside drinking less common modes include oral, Oral sex, blood products.

- Infective form is mature cyst passed in the faeces of convalescent and carriers.
- Cyst ingested through contaminated food, resist gastric acidity due to presence of cyst wall. In small intestine, trypsine lyse the cyst wall and quadrinucleate amoeba comes out. This stage is called as metacyst. Nuclei of metacyst immediately undergo division to form eight nuclei which gets mature to form 8 small amoebae.
- From small intestine trophozoite are carried to caecum where they lodge in the glandular crypts and undergo reproduction by binary fission. Some develop into precystic form and cysts which are then passed in feces to repeat the cycle.



Life cycle of E. histolytica

Note:
- Optimum habitat for metacystic trophozoites is the caecal mucosa.
- Cyst stage is found only in colon, not in extraintestinal sites.

Pathogenicity

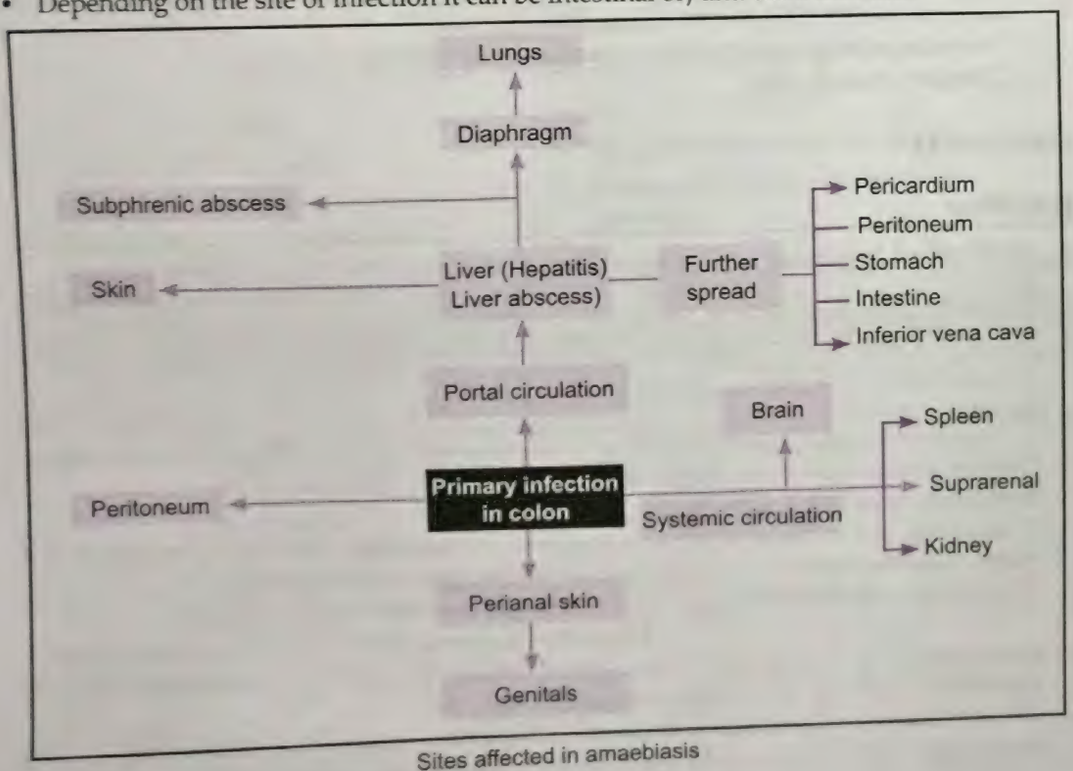
- In only 10% of total cases infected, intestinal invasion occurs, in rest of the cases entamoeba resides as commensal.
- Similarly not all strains of E. histolytical are pathogenic. Amoebic cysteine proteinase is a virulent factor which is present in pathogenic strains.
- Based on isoenzymes E. histolytica can be classified in to 22 zymodemes of these only 9 are invasive and rest are non-invasive commensals.
- It has been proposed to use the new species as E.dispar for non pathogenic entamoebae whereas E. histolytica for pathogenic ones.

I

Virulence factors
- Cystine Proteinase
- Amoebic Lectin
- Ionophore Proteins

Clinical Features

- Depending on the site of infection it can be intestinal or/and extra intestinal.



Sites affected in amaebiasis

I
- Most infections are asymptomatic

Diagnosis

- Definitive diagnosis of amoebiasis requires demonstration of actively motile trophozoite of entamoeba in freshly passed stool.

I

Amoebic Ulcer:
- Flask shaped ulcer with pin head centre and raised edges.
- Multiple
- Most numerous in caecum followed by sigmoido-rectal region

- Serology is helpful in diagnosis of extraintestinal amoebiasis, but not in intestinal amoebiasis.
  - Indirect hemogglutination (IHA) and latex agglutination (LA) are the sensitive serological test, whereas gel precipitation test are specific one.
- *DNA PCR* is the most sensitive and specific method for identifying E. histolytica.

Treatment

a. **Luminal amoebicides:** Diloxamide furoate, iodoquinol, paromomycin. Effective in amoebic colitis.
b. **Tissue Amoebicides:** Emetine, chloroquine, effective in extraintestinal amoebiasis Metronidazole is effective in both conditions.

ENTAMOEBA GINGIVALIS

A commensal amoeba found in mouth, of unhygienic human. Entamoeba gingivalis characteristically lacks cystic stage. Transmitted by direct oral contacts.

PATHOGENIC FREE LIVING AMOEBA

I

- Granulomatous Meningoencephalitis. Caused by Acanthamoeba (commonly) and Balamuthia. Usually seen in immunodeficient patient.
- Primary Amoebic Meningoencephalitis (PAM): Cause by free living amoeba naegleria.
- Chronic Amoebic Keratitis (CAK): Caused by Acanthamoeba.
- PAM and CAK usually affect healthy individual.

a. **Acanthamoeba:**
  - Causative agent of granulomatous amoebic meningoencephalitis and chronic amoebic keratitis.
  - **A. culbertsoni** is the ***most common*** species of acanthamoeba affecting human.
  - Infection is acquired by inhalation, ingestion or through traumatised skin.
  - Differs from naegleria in not having flagillar stage and in forming cyst in tissues.
b. **Naegleria:**
  - Causative agent of *primary amoebic meningoencephalitis*
  - Human infection is acquired by water containing cyst comes in, contact with nasal mucosa usually while swimming or diving.
  - Amoeba invades nasal mucosa and enters brain via olfactory nerve.
  - Disease is usually fatal.

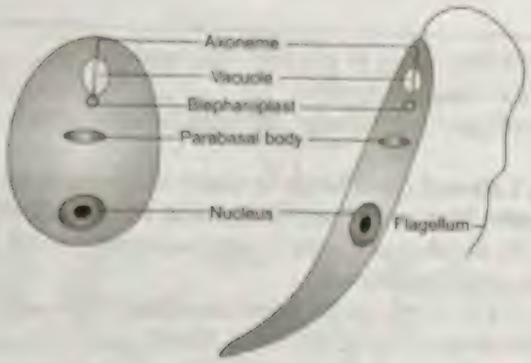
FLAGELLATES

GIARDIA

- An intestinal flagellates, and perhaps the most common intestinal protozoan pathogen.
  ...*Paniker 7/e 30*
- Lives in duodenum and jejunum
- Infection is acquired by ingestion of cyst in contaminated food and water.

LEISHMANIA

- Obligate intracellular hemo flagellate protozoan, causing kala-azar.
- Disease is vector borne with *sandfly* as *vector*.
- ***L. donovani*** is the causative agent of ***visceral leishmaniasis,*** whereas ***L. tropica*** is the major causative agent of cutaneous and/or ***mucocutaneous leishmaniasis.***
- L. donovani occurs in two forms: The amastigate form (LD body) in humans
  Promastigate form in sandfly and in artificial culture.
- Human acquires the infection by the bite of sandfly (P. argentipes). Most infections are sub-clinical, and only 3% develop kala-azar syndrome.
- ***I.P. is 2-6 months:*** Cutaneous lesion at the site of bite is usually absent in Indians
- **Pathology:**
  - Kala-azar results from reticuloendotheliosis, spleen is the organ most affected. Hepatomegaly may also be there
  - The resulting blockade of reticuloendothelial system results in marked depression of cell mediated immunity. As a response there is over production of immunoglobulins.



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Protozoan parasite found in lumen of small intestine: Giardia lamblia

- **Diagnosis:** Demonstration of amastigotes in smears of tissue aspirates is the gold standard for visceral leishmaniasis
- **Culture:** For culturing tissue material or blood NNN medium is adopted

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- Leishmania: Obligate intracellular protozoan

Treatment

- Sodium stibogluconate is the treatment of choice.

TRYPANSOMA CRUZI

- A hemoflagellate protozoan parasite, cause Chaga's disease
- Produce muscle fibrosis and destruct neural tissue that control tone of hollow organs thus produces myopathy, megaoesophagus, mega colon
- Intermediate host: Reduvid bug (vector)
- Definite host : Man
- In humans T. cruzi exists in both amastigote and trypomastigote form
- Infective stage: Metecyclic trypomastigotes
- Diagnosis: Demonstration of T. cruzi in blood on tissues on by serology

Chagas Disease

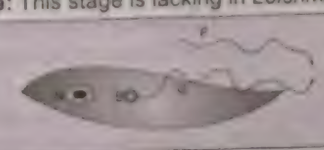
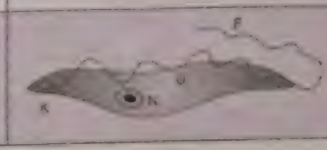
Acute

- Occurs in infant & children
- Present with fever, lymphadenopathy, splenomegaly and chagoma (localized swelling of skin)

Chronic

- Occurs in adults & elder age group
- Present with cardiac arrhythmias and dysphagia

Differences between various morphological stages of hemoflagellates

	Amastigote	Promastigote	Epimastigote	Trypomastigote
Morphological characteristics	Rounded or ovoid, without any external flagellum. The nucleus, kinetoplast, and axial filaments can be seen.	Lanceolate in shape. Kinetoplast is anterior to the nucleus (antenuclear kineloplast). There is no undulating membrane	Elongated, with the kinetoplast placed more posteriorly, close to and in front of the nucleus (juxtanuclear kinetoplast). Undulating membrane present	This stage is elongated, spindle shaped with a central nucleus. The kinetoplast is posterior to the nucleus (postnuclear kinetoplast) and situated at the posterior end of the body. Undulating membrane present
Seen in	*Trypanosoma cruzi* and Leishmania as intracellular form	It is the infective stage of *Leishmania*	It is the form in which *Trypanosoma brucei* occur in salivary gland of the vector tesetse fly and *Trypanosoma cruzi* in the midgut of the vector reduviid bug. **Note:** This stage is lacking in *Leishmania*	This is the infective stage of trypanosomes. **Note:** This stage is lacking in *Leishmania*
				

N: Nucleus; P: Parabasal body; B: Blepharoplast; A: Axoneme; U: Undulating membrane; F: Flagellum.

COCCIDIA

- Unicellular protozoa belonging to phylum *Apicomplexa*
- Toxoplasma gondii is the prototype
- ***Toxoplasma*** is an *obligate intracellular coccidian parasite* which completes its life cycle in two hosts:
  a. **Definitive host:** Cats and other feline in which both sexual and asexual cycle takes place
  b. **Intermediate hosts:** Man and other mammals in which only the asexual cycle takes place
- Life cycle can also be divided in to enteric and exoenteric cycle. Enteric cycle occurs in cat and other felines in small intestine.
- *Exoenteric cycle* occurs in humans and other mammals who acqiured infection after eating uncooked or undercoocked infected meat (lamb, park), ingestion of mature oocytes through food water or finger contaminated with cat feces; as congenital infection, through infected blood. Human infection is a dead end for parasite.
- Ingested sporozoites and bradyzoites multiply asexually to form tachyzoites which continue to multiply and spread locally by lymphatic system and blood.
- Some tachyzoites also spread to distance extraintestinal organs like brain, eye liner, spleen and skeleted muscles.

Clinical Features

- **Immunocompetant:** Mostly asymptomatic commonest manifestation is lympha denopathy (cervical area is most commonly affected)
  - In eyes (occular toxoplasmosis) is present as uveitis, choroiditis, or chorioretinitis
- **Immunocompomised:** Involvement of brain is most common (clinically present as encephalitis, altered mental status).
- Congenital toxoplasmosis

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Congenital parasitic infection
- Toxoplasma gondii
- Plasmodium spp.
- Trypanosoma cruzi

PLASMODIUM

- Causative agent of malaria.
- Four species of plasmodia cause malaria in man viz: P. vivax, P. falciparum, P. malaria, and P. ovale. P vivax and P. falciparum being the most common.
- Discovered by Alphonse Lavaran, a French Army surgeon. Ronald Ross established the mode of transmission of disease in Secunderabad India. Both of them were awarded Nobel Prize.
- **Vector:** In human malaria is transmitted by the female anopheles mosquito.

Note: In P. vivax and P. ovale infections, a proportion of the intrahepatic form do not divide immediately but remain dormant for a period ranging from 3 weeks to a year or longer. These dormant forms, or "hypnozoites" are the cause of the relapses that characterize infection with these two species.

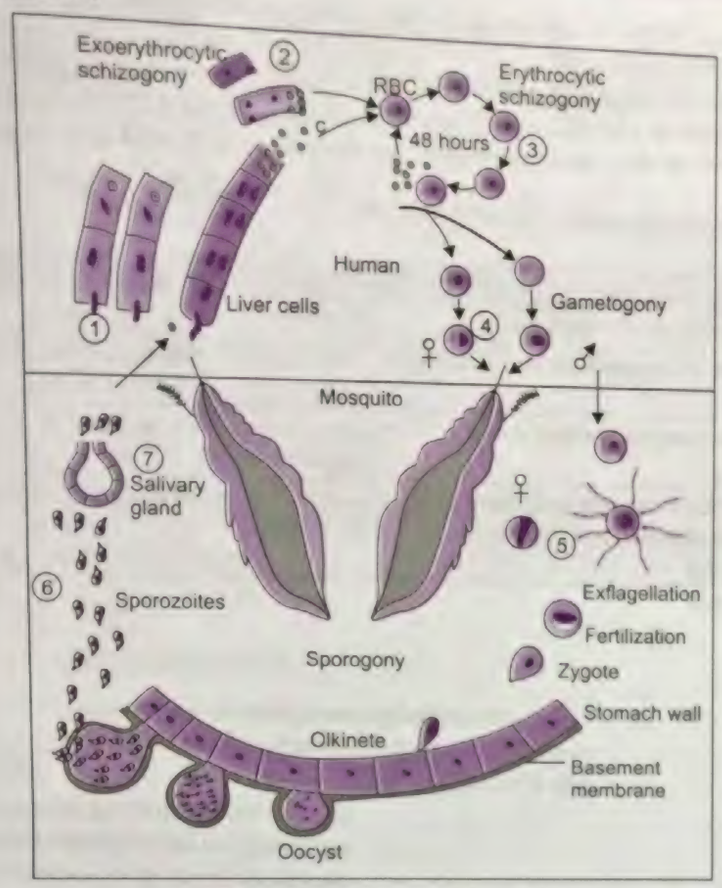
Person with gametocyte in blood is a carrier of reservoir of malaria.

Pathogenesis and Clinical Features

- Merozoites released after rupture of hepatocytes, enters in the RBC.
- Glycophorin acts as receptor for merozoites in RBC.
- Parasite feeds on the hemoglobin of the erythrocyte; however it does not metabolise hemoglobin completely and leaves behind a haematin-globin pigment called as malaria pigment ***(also called as haemozoin pigment)*** Rupture of mature schizont release huge variety of pyrogens which are responsible for febrile response of malaria. Clinically presenting as:
  - Fever (MC), myalgia, headache
  - Seizures can be seen with any malaria, but generalized seizure are specifically associated with falciparum malaria.
  - Coma is characteristic and ominous feature of falciparum malaria.

Note: Duration of erythrocytic schizogony varies according to plasmodium species (48 hour is falciparum, ovole and vivax, 72 hrs. in malariae), pre-erythrocytic schizogony doesn't produce clinical illness.

Life Cycle of Plasmodium



Life cycle of Plasmodium vivax

1. Sporozoites from the salivary gland of female mosquito are injected into skin capillaries when the mosquito bites humans.
2. They enter liver cells to initiate exoerythrocytic schizogony to form merozoites which infect red blood cells, to initiate the cycle of erythrocytic schizogony which is repeated every 48 hours.
3. Some merozoites initiate gametogony, forming male and female gametocytes.
4. Mosquito ingests gametocytes in its blood meal. Male gametocyte undergoes exflagellation.
5. One male, gamete fertilises female gamete to form zygote. It develops into the motile ookinete, which penetrates the stomach wall and becomes the oocyst inside which sporozoites develop.
6. Sporozoites released by rupture of mature oocyst enter the haemocoele and reach the salivary glands of the mosquito.

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- A sexual stage: Human
- Sexual stage: Mosquito
- P. vivax and P. falciparum are the commonest cause of malaria in India.

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Protozoa detected in blood:

- Trypanosoma
- Plasmodium
- Babesia
- Leishmania

Black waters Fever

- Sometimes seen in falciparum malaria, particularly in patients who experienced repeated infections and inadequate quinine treatment.
- Clinical features include bilious vomiting and prostration with passage of dark red and blackish urine.
- Pathogenesis include massive intravascular hemolysis secondary to anti-erythrocyte antibodies.
- Complications include renal failure, acute hepatic failure and circulatory collapse.

Algid Malaria

- Malaria characterized by peripheral circulatory failure, rapid thready pulse with low BP and cold clammy skin.

Septicemic Malaria

- High continuous fever with dissemination of parasite to various organs leading to multi-organ failure.

Diagnosis

a. **Demonstration of parasite:** Demonstration of asexual form of parasite in stained peripheral blood *smear is gold standard*. Both thick and thin smears should be examined.

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P. facliparum: Malignant tertian = Pernicious malariae
P. Ovale: Ovale tertian
P. vivax: Benign tertian
P. malariae: Quartan malaria

- *Thick film has the advantage of concentrating the parasite* (by 40 to 100 fold), thus enhance the diagnostic sensitivity.

b. **Antibody based Methods:** Sticks or card tests that provide rapid detection of P. falciparum, specific, histidine rich protein (PjHRP-2), or LDH antigens in finger prick method. However they remain positive for several weeks after acute infection.

Note: Recrudescence vs relapse Table 6.5 pNo. 79.

Recrudescence	Relapse
Seen in P. *falciparum* and P. *malariae*	Seen in P. *vivax* and P. *ovale*
Due to persistence of the parasite at a subclinical level in circulation	Due to reactivation of hypnozoites present in liver cells
Occurs within a few weeks or months of a previous attack	Occurs usually 24 weeks to 5 years after the primary attack
Can be prevented by adequate drug therapy or use of newer antimalarial drugs in case of drug resistance	Can be prevented by giving primaquine to eradicate hypnozoites

Multiple Choice Questions

Amebae

1. **All are true about entamoeba histolytica except:** [AIIMS Nov 2014]
   a. Stool trophozoites are essential for diagnosis
   b. Mostly asymptomatic
   c. Cause disease in brain, liver
   d. Infection does not provide immunity

2. **Which of the following is true regarding the trophozoite of *E. histolytica*?** [AI 96]
   a. Has eccentric karyosomes
   b. Presence of bacteria inside
   c. Has four nuclei
   d. Shows erythrophagocytosis

3. **Acute primary amoebic meningoencephalitis true is:**
   a. Meningitis caused by *Acanthamoeba* species is acute in nature [AIIMS 08]
   b. Diagnosed by trophozoite in CSF
   c. Caused by feco-oral transmission
   d. More common in tropical climate

4. **A patient presents with lower gastrointestinal bleed. Sigmoidoscopy shows ulcers in the sigmoid. Biopsy from this area shows flask-shaped ulcers. Which of the following is the most appropriate treatment?**
   a. Intravenous ceftriaxone [AIIMS 05]
   b. Intravenous metronidazole
   c. Intravenous steroids and sulphasalazine
   d. Hydrocortisone enemas

5. **Invasive amoebiasis can be best diagnosed by:**
   a. ELISA [AIIMS 01]
   b. Counter current immunoelectrophoresis
   c. Indirect hemagglutination test
   d. Complement fixation test

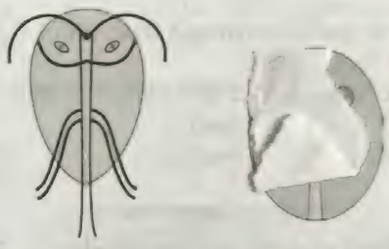
6. **True about amoebic colitis is:** [PGI 02]
   a. Caused by *E. histolytica*
   b. Cyst contains 8 nuclei
   c. Flask-shaped ulcers are present
   d. Cecum is most commonly affected
   e. Is premalignant

7. **A 30-year-old patient treated with features of acute meningoencephalitis in casualty. His CSF on wet mount microscopy revealed motile unicellular microorganisms. The most likely organism is:** [AIIMS 05]
   a. *Naegleria fowleri*
   b. *Acanthamoeba castellani*
   c. *Entamoeba histolytica*
   d. *Trypanosoma cruzi*

8. **Transmission of amoebiasis occurs by all except:** [AIIMS Nov 10]
   a. Cockroach
   b. Feco oral
   c. Oro rectal
   d. Vertical transmission

Flagellates

9. **An anxious mother brought her 4-year-old daughter to the pediatrician. The girl was passing loose bulky stools for the past 20 days. This was often associated with pain in abdomen. The pediatrician suggested the stool examination, which showed the following organism. Identify the organism:** [AI 03]



   a. *Entamoeba histolytica*
   b. *Giardia lamblia*
   c. *Cryptosporidium*
   d. *E. coli*

10. **A patient present with diarrhoea. Analysis of stool on wet mount shows mobile protozoa without RBCs and pus cells. The diagnosis is:** [AI 00]
    a. *Balantidium Coli*
    b. Giardiasis
    c. *Trichomonas hominis*
    d. *Entamoeba histolytica*

11. **Vector of Kala-azar is:** [AIIMS 07]
    a. Flea
    b. Tsetse fly
    c. Sand fly
    d. Mite

12. **Parasite causing encephalitis is/are:** [PGI 04]
    a. *Entamoeba histolytica*
    b. *T. gondii*
    c. *Angiostrongylus cantonensis*
    d. *T. cruzi*

13. **Reduvid bug is a vector for the transmission of:** [AIIMS 05]
    a. Relapsing fever
    b. Lyme's disease
    c. Scrub typhus
    d. Chaga's disease

14. **Which of the following infestations leads to malabsorption?** [AI 06; AIIMS 04]
    a. *Giardia lamblia*
    b. *Ascaris lumbricoides*
    c. *Necator americana*
    d. *Ancylostoma duodenale*

15. **Recurrent giardiasis is associated with:** [AIIMS 00; 97]
    a. Severe combined immunodeficiency
    b. Common variable immunodeficiency
    c. Digeorge syndrome
    d. C8 deficiency

16. **Which of the following is true about *Giardia lamblia*?**
    a. Malabsorption commonly seen **[PGI 05]**
    b. Trophozoite form is binucleate pear shaped
    c. Diarrhea is seen
    d. Jejunal wash fluid is diagnostic
    e. Is a free living nematode

17. **Visceral leishmaniasis:** **[PGI 00]**
    a. Caused by *L. tropica*
    b. Post-leishmaniasis dermatitis is common
    c. Antimonial are useful drugs
    d. Diagnosed by blood smear
    e. Vector is *Phlebotomus sargenti*

18. **"Amastigote forms" are seen in:** **[PGI 01]**
    a. *Leishmania donovani* b. *Toxoplasma gondii*
    c. *Leishmania major* d. *Entamoeba*

19. **Mucocutaneous leishmaniasis is caused by:** **[PGI 97]**
    a. *L. braziliensis* b. *L. tropica*
    c. *L. donovani* d. *L. orientalis*

20. **Which of the following is true about *Giardia*?**
    a. CFT is diagnostic **[PGI 95]**
    b. Trophozoites and cysts are seen in man
    c. Lives in lower intestine
    d. Invades normal mucosa

Sporozoa

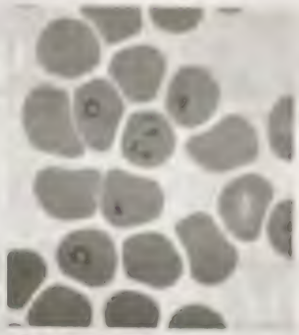
21. **True about visceral leishmaniasis:** **[PGI May 2013]**
    a. Neutropenia
    b. Eosinophilia
    c. Hyper gamma globulinemia
    d. Skin hyperpigmentation
    e. Lymphadenopathy

22. **All of the following statements about toxoplasmosis are true except:** **[AI 01]**
    a. Oocyst in freshly passed cat's faeces is not infective
    b. May spread by organ transplantation
    c. Maternal infection after 6 months has high risk of transmission
    d. Arthralgia, sore throat and abdominal pain are the most common manifestation

23. ***Plasmodium falciparum* infection of man is characterized by:** **[AI 97]**
    a. The erythrocytes are increased in size
    b. All stages of erythrocytic schizogony are seen in peripheral blood
    c. Multiple infection of erythrocytes are seen
    d. Each erythrocytic cycle lasts 72 hours

24. **Which of the following is true about malaria?** **[AI 96]**
    a. Size of RBC is enlarged in *vivax* infection
    b. Size of RBC is enlarged in *falciparum* infection
    c. Schuffner's dots are seen in *malariae* infection
    d. Relapse is seen in *falciparum* infection

25a. **A 15-year-old boy presented with fever and chills for 3 days. On examination he was found to have delayed skin pinch time and dry oral mucosa. A peripheral blood smear revealed the following picture. Identify the pathogen involved:**



    a. Plasmodium falciparum **[AIIMS 16]**
    b. Babesia
    c. Plasmodium vivax
    d. Salmonella typhi

25b. **Why are schizont and late trophozoite stages of Plasmodium falciparum not seen in peripheral blood smear?**
    a. They are sequestered in the spleen **[AIIMS 16]**
    b. Due to adherence to the capillary endothelium, they are not seen in peripheral blood
    c. Due to antigen-antibody reaction and removal
    d. They are seen in mosquito blood

25c. **Which of the following is true about *P. falciparum*?**
    a. James dots are seen **[AI 96]**
    b. Accole forms are seen
    c. Relapse are frequent
    d. Longest incubation period

26. **Which one of the following is detected by the antigen detection test used for the diagnosis of *P. falciparum* malaria?**
    a. Circum sporozoite protein **[AIIMS 04]**
    b. Merozoite surface antigen
    c. Histidine rich protein I (HRPI)
    d. Histidine rich protein II (HRP II)

27. **Which of the following statement is false?** **[AIIMS 03]**
    a. The presence of ingested erythrocytes is seen only in *Entamoeba histolytica*
    b. Young adult male of low socioeconomic status are most commonly affected by invasive amoebiasis
    c. A low iron content in the diet predispose to invasive amoebiasis
    d. The pathogenic and non pathogenic strains of *E. histolytica* can be differentiated by the electrophoretic study of zymodemes

28. **Toxoplasmosis in the foetus can be best confirmed by:** **[AIIMS 02]**
    a. IgM antibodies against *Toxoplasma* in the mother
    b. IgM antibodies against *Toxoplasma* in the foetus
    c. IgG antibodies against *Toxoplasma* in the mother
    d. IgG antibodies against *Toxoplasma* in the foetus

29. **True about toxoplasmosis is all except:** **[AIIMS 01]**
    a. In adults toxoplasmosis is usually asymptomatic
    b. IgG antibodies are diagnostic in congenital toxoplasmosis
    c. Is a anthroponotic disease
    d. Encephalitis is uncommon (rare) in immunocompetent individuals

30. **A 35-year-old male suffering from sudden onset of high grade fever. On malarial slide examination all stages of parasites are seen with schizonts of 20 microns size with 14-20 merozoites per cell and yellow brown pigment. The diagnosis is:**
    a. *Plasmodium falciparum* **[AIIMS 01]**
    b. *Plasmodium vivax*
    c. *Plasmodium malariae*
    d. *Plasmodium ovale*

31. **True about Toxoplasma gondii:** **[PGI 11]**
    a. Direct spread by blood/urine is main mode of transmission
    b. Cerebellum is MC site of brain involvement
    c. Isolation of parasite from blood is very easy
    d. Laboratory test are useful for making diagnosis
    e. Infection is severe and progressive in immunocompromised host

32. **Stages seen in peripheral smear of *falciparum* malaria:**
    a. Schizonts b. Gametocytes **[PGI 05]**
    c. Accole trophozoite d. Ring form

33. ***P. falciparum* causes:** **[PGI 05]**
    a. Thrombocytopenia b. DIC
    c. Hemolysis d. Hematemesis

34. **True about toxoplasmosis:** **[PGI 03]**
    a. Due to ingestion of sporocyst with meat
    b. Due to ingestion of oocyst from cat's faeces
    c. Spiramycin given in pregnancy
    d. Due to bite of Anopheles mosquito
    e. Mostly symptomatic

35. **True about babesiosis:** **[PGI 03]**
    a. Caused by *Babesis microti*
    b. Resides in RBC
    c. Resides in WBC
    d. Chloroquine is drug of choice
    e. It is a filarial parasite

36. **True about *Cryptosporidium parvum:*** **[PGI 03]**
    a. Affect only in immunocompromised patient
    b. It is one of the common opportunistic infection in AIDS
    c. Cyst size 12-15 mm
    d. AFB +ve cyst
    e. Treatment is metronidazole

37. **Chronic complication of malaria:** **[PGI 02]**
    a. Splenomegaly b. Nephrotic syndrome
    c. Pneumonia d. Hodgkin's disease

38. **True about malaria diagnosis:** **[PGI 00]**
    a. Thick smear to identify parasite
    b. ABER reveals positivity by 100
    c. All have same incubation
    d. Fluorescein Ab becomes positive within 1 week

39. ***Acid fast* organism with oocyte of size 5 μm on stool examination, causing diarrhoea in HIV positive patient:** **[AIIMS May 09]**
    a. *Cryptosporidium* b. *Isospora belli*
    c. *Microsoporidia* d. *Blastocystis hominii*

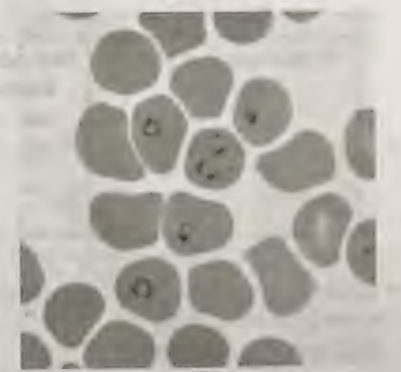
40. **Which is the infective stage for mosquito in case of plasmodium vivax:** **[AIIMS Nov 09]**
    a. Gametocyte b. Sporozoite
    c. Zygote d. Merozoite

41. **Which is not true about fluorescent antibody detection test for diagnosis of *falciparum*?**
    a. It is a immunochromatographic test **[AIIMS 08]**
    b. Detects aldolase antigens
    c. Detects LDH antigens
    d. Detects histidine rich proteins 1

42. **Congenital toxoplasmosis not true:** **[AIIMS May 09]**
    a. IgA is more sensitive than IgM
    b. An increase in IgM beyond 1st week of life is diagnostic
    c. IgG is diagnostic
    d. Dye test is used

43(a). **In HIV patient with malabsorption, fever, chronic diarrhea, with acid fast positive organism, What is causative agent?** **[AIIMS May 10]**
    a. Giardia b. Microsporidia
    c. Isospora d. E. histolytica

43(b). **The following diagram depicts blood smear of which species?** **[AIIMS Nov 2015]**
    a. P. vivax b. P. falciparum
    c. P. ovale d. P. malariae



Miscellaneous

44. **Parasitic encephalitis is caused by:** **[PGI 05]**
    a. *Ascaris* b. *Naegleria*
    c. *Acanthamoeba* d. *Balamuthia*
    e. *Entamoeba*

45. **About microsporidia all of the following are false except:**
    a. It is a fungus b. It is a protozoa **[PGI 05]**
    c. It is a bacteria d. It is trematoda
    e. It is associated with diarrhoea in HIV patients

46. **Eosinophilic meningoencephalitis is caused by:**
    a. *Ganthostoma spiralis* b. *Naegleria* **[PGI 00]**
    c. *Toxocara canis* d. *Angiostrongylus cantonensis*

47. **A patient complaints of diarrhea, stool examination shows ova of size <100 μm, which of the following can not be the cause:** **[AIIMS May 2013]**
    a. Cryptosporidium b. Opisthorchis viverni
    c. Isospora d. E. histolytica

Explanations and References with Illustrative Answers

1. Ans. (a) Stool trophozoites are essential for diagnosis *Ref. Paniker 7/e, p 21*

Diagnosis of intestinal amoebiasis is suggested by presence of cyst or dead trophozoite in stool.

A. Laboratory diagnosis of Intestinal amoebiasis

Stool examination	Stool Culture Media used	Mucosal scrapings	Serodiagnosis	Molecular diagnosis
• Microscopy • Macroscopy • Iodine stained preparation preparations to demonstrate trophozoite or cyst	• Boeck and Drbohlav • NIH polygenic • Craig's • Nelson's • Robinson's	• Wet mount Stained preparation	• IHA • ELISA • Latex agglutination test	• DNA probe

B. Laboratory diagnosis of amoebic liver abscess

Microscopy of pus or aspirate	Histopathological examination of pus or aspirate	Serodiagnosis	Radiological examination	Stool examination
		• IHA • ELISA • Latex agglutination test	• X-ray • USG • CT scan • MRI	

Note: Stool culture is a sensitive method in diagnosing chronic and asymptomatic amoebiasis

Media used for stool culture:

- Boeck and Drbohlav Media
- NIH polygenic media
- Craig's Media
- Nelson's Media
- Robinson's Media

2. Ans. (d) Shows erythrophagocytosis *Ref. Paniker 7/e, p 24*

Differential features of intestinal entamoebe			
Features	*E. histolytica*	*E. hartmanni*	*E. coli*
Trophozoite			
Size (μ m)	12-60	4-12	20-50
Motility	Active	Active	Sluggish
Pseudopodia	Finger shaped, rapidly extruded	Finger shaped, rapidly extruded	Short, blunt, slowly extruded
Cytoplasm	*Clearly defined into* endoplasm and ectoplasm	Clearly defined into ectoplasm and endoplasm	Not defined
Inclusions	*Red blood cells present,* no bacteria	Bacteria and other particles, no red blood cells	Bacteria and other particles, no red blood cell
Nucleus	Not clearly visible in unstained films; It is *eccentric*	Not clearly visible in unstained films	Visible in unstained films

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Differential features of intestinal entamoebe			
Features	*E. histolytica*	*E. hartmanni*	*E. coli*
Karyosome	Small, *central*	Small, eccentric	Large, *eccentric*
Nuclear membrane	Delicate, with *fine chromatin dots*	Coarse chromatin granules	Thick, with coarse chromatin
Cyst			
Size (μm)	10-15	5-10	10-30
Nuclei in mature cyst	4, central karyosome	4	8, eccentric karyosome
Glycogen mass	Seen in uninucleate, but not in quadrinucleate stage	Seen in uninucleate, but not in quadrinucleate stage	Seen upto quadrinucleate stage
Chromatidial bars	1-4, with rounded ends	Often numerous, shape irregular	Splinter like with angular ends

3. Ans. (b) Diagnosis by trophozoite in CSF *Ref. Paniker 7/e, p 25 - 26; Harrison 19/e, p 1367*

Acute primary amoebic meningoencephalitis (PAM) is caused by Naegleria fowleri

Naegleria

NAEGLERI FOWLERI: It has 3 stage -
(Brain eating amoeba)

Amoeboid trophozoite form

Pear shaped flagellated form
(main infective stage)

Dormant cyst form
(Outside the body)

Route of Transmission

- It is seen in fresh water lake.
- Aspiration of water contaminated with trophozoites or cysts or inhalation of cyst leading to invasion of olfactory neuroepithelium, then into meninges and brain.

Clinical features

- ***Commonly seen*** in ***other wise*** healthy children or young adults who often report recent swimming in lakes or heated swimming pools.
- Parosmia/anosmia, headache, high fever, nausea, vomiting, meningismus.
- Photophobia and palsies of 3rd, 4rh and 6th cranial nerves are common.
- Seizures, coma and most patient die within a week.

Diagnosis

- Detection of motile trophozoites in wet mounts of fresh spinal fluid.
- Bacterial meningitis *without any bacteria on* Gram's staining/antigen detection assay and culture.

Treatment

- Amphotericin
- Rifampicin may added.

Remember: Other Pathogenic free living amoebae

i. Acanthamoeba spp. – Causing chronic granulomatous amoebic encephalitis (GAE), chronic amoebic keratitis (associated with use of contact lens)

ii. Balamuthia – Cause meningoencephalitus in both immunocompromised and immunocompetent host particularly children and adult.

4. Ans. (b) Intravenous metronidazole *Ref. Harrison 17/e, p 1278, 18/e, p 1686, 19/e p 1366*

It is case of intestinal amoebiasis in the form of amoebic dysentry.

Drug Therapy for Amebiasis		
Asymptomatic carrier (Luminal agents)	**Acute colitis**	**Amebic liver abscess**
• Iodoquinol • Paromomycin	• Metronidazole plus Luminal agent	• Metronidazole or Tinidazole or tinidazole or ornidazole plus Luminal agent

5. **Ans. (a) ELISA** *Ref. Harrison 19/e, p 1366 - 1367, 18/e, p 1685; Paniker 7/e, p 21*

Diagnosis of Amoebiasis

Specimen

- Fluid feces for examination of trophozoite.
- Formed feces for cysts.
- Scrapings and biopsies – ***most commonly*** by colonoscopy.
- Liver abscess aspirate for trophozoites *(as cyst are absent in tissues)* from edge of abscess, not the necrotic center.
- Blood for serologic test and cell counts.

i. ***Fecal findings suggestive of amoebic colitis*** - positive test for heme, paucity of neutrophils, ***amoebic cyst or hematophagous trophozoite*** *(definitive)*. Examine at least 3 fresh stool specimen.

ii. ***Culture*** - Diphasic Locke-egg, Monophasic TYGSM and Robinson's media are used.

iii. ***Trophozoites in biopsy*** specimen from colonic mass confirm the diagnosis of amoeboma.

PCR assay for entamoeba DNA is most sensitive and specific method for identifying E. histolytica

Stool diagnostic test based on the detection of the Gal/GalNAC lectin of E.histolytica compare favorably with the PCR

Harrison 19/e, p 1366

iv. ***Serology: (Becomes positive only in invasive amoebiasis)***

- Is primarily for extra-intestinal amoebiasis when stools are often negative.
- *Most commonly* done by indirect hemagglutination assay (IHA) but it can't distinguish recent from past infection since it remains positive for as long as 10 years.
- ELISA and agar gel diffusion assay are positive in more than 90% of patients with colitis, amoebomas or liver abscess.
- *MC* test done for invasive amoebiasis – *IHA*
- Antamoebic antibodies occur only with *E. histolytica* not with other *Entamoeba*.
- Enzymeba test is based on finding of histolysain (major cysteine protease of virulent form) in the intestine (stool) plus circulating antibodies to histolysain after tissue invasion.

- Enzymeba and ELISA helps distinguish pathogenic from non-pathogenic strain (*E. dispar*) in a stool specimen.

v. ***Radiation methods*** – Hepatic abscess usually shows elevation of right dome of diaphragm by US, CT, MRI, etc.

vi. ***Liver enzyme*** are normal or minimally elevated (alkaline phosphatase most often elevated) even with large liver abscess.

6. **Ans. (a, c) and (d) Caused by *E. histolytica*, Flask-shaped ulcers are present and Cecum is most commonly affected**

Ref. Paniker 7/e, p 15; Jawetz 27/e, p 711

E. histolytica – It has 3 stages:

a. **Trophozoite or Vegetative or Amoeboid form**
   - It is the only form present in tissue.
   - Growing feading stage and is activity motile
   - It can't initiate infection.

b. **Pre-cystic stage**
   - Encystment occur in intestinal lumen not in feces or not in tissues.
   - Not contain RBC and other ingested food particles.

c. **Cystic stage**
   - Mature Quadrinucleate cyst: *Infective stage*
   - It is present *only in lumen* of colon and in mushy or formed feces.
   - When stained with iodine, nuclear chromatin and karyosome appears bright yellow while chromidial bars are unstained.

Pathogenesis

8 small trophozoites (=amoebulae or metacystic trophozoites) are released per infective cyst causing:

Primary amoebiasis	Secondary intestinal lesion	Chronic infection	Invasive amoebiasis
• ***MC*** site caecum	• ***MC*** in cecum, appendix or nearby ascending colon	• Occurs when organism travel to ileocaecal valve and terminal ileum	• Extraintestinal infection is metastatic through portal circulation
• Lesion with Pinhead sized center and raised edges	• It occurs due to extension from primary lesion	• Sigmoid colon and rectum are favoured site • Amoeboma may form	• MC form is amebic hepatitis or liver abscess
• Flask shaped ulcers with narrow neck and broad base			
• Mucosa surface between ulcer is normal			
• Ulcer is not premalignant			

- Intestinal amoebiasis present usually as amoebic dysentery.
- Contents of amoebic abscess is called as *anchovy paste.*
- *MC* complication of amoeba liver abscess is *Pleuropulmonary involvement.*

7. **Ans. (a)** ***Naegleria fowleri*** *Ref. Paniker 7/e, p 26 - 27; Harrison 19/e, p 1367, 18/e, p 1686*

It is a typical presentation of meningitis caused by Naegleria fowleri.

Let's consider other options:

Acanthamoeba

- No flagellated stage
- Trophozo ⇌ Cyst (Formed in tissue) ***(Infective stage)***
- Encephalitis occurs typically in chronically ill or debilitated patient (lymphoproliferative disorder, chemotherapy, etc) and features of CNS lesion often mimics space occupying lesion.
- Infection reaches the CNS hematogenously from primary focus in the sinuses, skin nodules/ulcers and lungs.

Diagnosis:

- Demonstration of trophozoites and cyst on wet mount of CSF.
- Culture on non-nutrient agar plates seeded with *E.coli.*

Entamoeba histolytica

Brain may occasionally involve (<0.1%), result from hematogenous spread from amoebic lesions of colon.

... Harrison 19/e, p 1365

Trypanosoma cruzi

Neurologic sign are not common but meningoencephalitis have been reported especially in children < 2 years old.

... Harrison 18/e, p 1717

So, from above description it is clear that patient of 30 year (young adult with no chronic disease and no GI symptoms) with meningoencephalitis and motile unicellular (all protozoan are unicellular) microorganism on wet mount; is a typical case of PAM of Naegleri fowleri.

8. **Ans. (D) Vertical transmission** *Ref. Paniker 6/e, p 14, 16*

Epidemiological features of amoebiasis

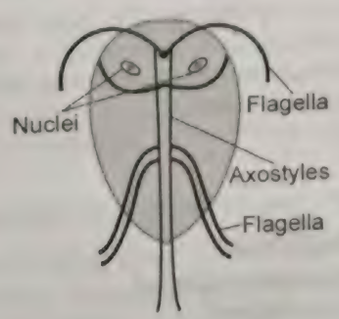
Adults > children
Males > females
Infective stage–Quadrinucleate cyst
Source of infection – carrier or asymptomatic cyst passer

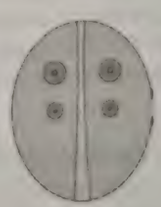
Route of transmission	–	Fecoral
Mode of transmission	–	Contaminated food and water
Mechanical vectors	–	Flies and cockroaches
Sexual transmission	–	Occur in homosexuals (*gay bowel disease*)

Remember: Parasites transmitted transplacentally (vertical transmission) are:
Toxoplasmosis
Plasmodium
T. Cruzi

9. **Ans. (b) Giardia lamblia** *Ref. Paniker 7/e, p 30*

It is typical figure of ***trophozoite and cyst of Giardia lamblia*** which is the *MC* intestinal protozoan parasite.





Trophozoite		Cyst	
•	Pyriform/Heart/Tennis or badminton racket shaped	•	Encystation occur in colon
•	Bilateral symmetrical and has large concave sucking disc; 2 axostyles; 2 parabasal or median bodies; 4 pairs of flagella; **2 nuclei** with prominent central karyosomes	–	Ovoid with hyaline cyst wall
		–	Mature cyst has ***4 nuclei (= infective stage)***
		–	Passed in stool and ***is infectious***
•	Divides by longitudinal binary fission	–	Infective dose is as few as 10 cyst
•	Passed in stool but ***not infectious***		
•	Motility resemble *"falling leaf"*		

10. Ans. (b) Giardiasis *Ref. Paniker 7/e, p 30, 107, 112; Jawetz 25/e, p 670- 672, 27/e p 710*

Let consider each option one by one.

a. *Balantidium coli*
- Live in large intestine causing balantidiasis (zoonosis).
- Occurs in 2 stages; trophozoite (binucleated) and cyst (= infective form).
- Clinical disease results only when trophozoites burrow into mucosa causing ulcer and submucosal abscesses resembling lesion of amoebiasis so it 'resembles amoebiasis' causing diarrhoea or frank dysentery, so stool may contains pus cells.
- Treatment: Tetracyline/metronidazole/nitroimidazole

b. *Giardia lamblia*
- Intestinal flagellate, the only protozoan parasite found in the lumen of human small intestine (Duodenum and upper jejunum).
- Infection is acquired by ingestion of cysts in contaminated food and water.
- It does not invade tissue (so no RBC and pus cells in faeces) but remains attached to epithelial surface by sucking disc which may cause abnormalities of villous architecture and loss of brush border enzymic activities leading to self limited mucus diarrhoea; malabsorption like syndrome; weight loss; abdominal cramps. Occasionally cause biliary colic and jaundice. *... Paniker 6/e, p 38*
- **Diagnosis:** Detection of cysts (encystation occur in colon) as well as trophozoite (die outside so not infectious) in stool sample or in duodenal aspiration by enterotest if biliary symptoms predominate.
- Detection of antigen by ELISA or immunochromatographic strip test.
- Giardiasis is a cause of traveller's diarrhoea.
- **Treatment:** *DOC* – Metronidazole/tinidazole
  Alternative – quinacrine hydrochloride and furazolidone.

c. *Trichomonas vaginalis*
- Not cause diarrhoea.

d. *Entamoeba histolytica*
- It cause amoebic dysentery so stool contain cellular exudate as well as RBC, charcot leyden crystals, amoeba, etc.

11. Ans. (c) Sand fly *Ref. Harrison 19/e, p 1389, 18/e, p 1708; Paniker 7/e, p 51 - 54*

Visceral Leishmaniasis/Kala-azar

"Beside the bite of Sand fly (P. argentipes), it is also transmitted by blood transfusion, sexual contact, inoculation and congenitally."

Important points about visceral Leishmaniasis/Kalazar
- Caused by *L. donovani:*
- *MC* organ affected: **spleen.**
- Progressive emaciation, irregular fever sometimes hectic, hepatosplenomegaly, bone marrow infiltration epistaxis, bleeding, cancrum oris, pneumonia may occur.
- Some develops post-kalaazar dermal leishmaniasis (PKDL) characterized by skin lesion mostly on face.
- Usually develop about an year on two, after recovery from systemic illness
- PKDL occur in 10-20% cases and it is seen mainly in India.
- Relapse of visceral lesion can also occur.

Diagnosis
- *Specimen:* Peripheral blood – *Best* to examine buffy coat.
  Bone marrow aspirate – *MC* diagnostic specimen collected.
  Spleen aspirates – Diagnostic yields is highest.

1. *Demonstration of parasites by:*
   - Microscopy [LD bodies i.e. amastigote seen within macrophages].
   - Culture in NNN or Tobie's medium shows only promastigote while in Schneiders's liquid tissue culture amastigote form also seen.
   - Animal inoculation – Hamster is the animal employed.
2. *Demonstration of antibodies by* using specific (direct agglutination test, CFT etc) or non-specific (WKK) antigen.
3. *Nonspecific serum test* (Napier's aldehyde or Formol gel test and Chopra's antimony test) ***based on increased globulin content*** of serum.
4. *Absence of hypersensitivity to* leishmanial antigen, i.e. Montenegro (leishmanin) skin test is negative in kala-azar.
5. *Others lab findings* – Anemia, leucopenia, neutropenia, thrombocytopenia with hypergammaglobulinaemia and reversal of albuminglobulin ratio.

Treatment:
- First line therapy
  - Pentavalent antimony
  - Amphotericin B lipid formulation
- Alternative
  - Amphotericin B (deoxy cholate), paromomycin sulfate, pentamidine isethionate, miltefosine.

12. Ans. (b, c) and (d) *T. gondii, Angiostrongylus cantonensis* and *T. cruzi* *Ref. Harrison 17/e, p 2632; 16/e, p 1239, 1245*

Parasites causing encephalitis are:

- *Toxoplasma gondii* (rarely)
- *Trypanosoma cruzi* (occasionally)
- *Angiostrongylus cantonensis* (eosinophilic meningoencephalitis)
- *Acanthamoeba* (Granulomatous amoebic encephalitis)
- *Naegleria fowleri* (Primary amebic meningoencephalitis)
- *Balamuthia.*

13. Ans. (d) Chaga's disease *Ref. Paniker 7/e, p 46; Park 22/e p724*

Hemoflagellate		Vector	Disease	Infective stage for man
Trypanosomes				
i.	*T. brucei gambiense and T. brucei rhodesiense*	Tse-Tse fly	African trypanosomiasis (sleeping sickness)	Metacyclic trypomastigote by inoculative route of fly
ii.	*T. cruzi* (Intracellular)	Reduvid bug or triatomine bug	Chaga's disease (South American trypanosomiasis)	Metacyclic trypomastigote by rubbing faces into wound made by bite of bug (Stercorarian transmission)
Leishmania – In man, amastigote form present in macrophage forming LD (Leishmania donovani) body				
i.	*L. donovani*	Female Sandfly (P. argentipes)	Visceral leishmaniasis (= kala azar)	Promastigote form by bite of fly
ii.	*L. Tropica*	P. sargenti and P. papatasi	Cutaneous leish-maniasis (=oriental sore)	Promastigote form by bite of fly
iii.	*L. braziliensis*	Sandfly	Mucocutaneous leishmaniasis	Promastigote form by bite of fly

14. Ans. (a) *Giardia lamblia* *Ref. Ghai 6/e, p 252; Harrison 19/e, p 1405, 18/e, p 1730*

Remember:
- *Ascaris lumbricoides* in small intestine usually cause no symptoms. In children it may cause pain and intestinal obstruction sometimes complicated by perforation, intussusception or volvulus.
- Migration to aberrant site can cause biliary colic, cholecystitis, cholangitis, pancreatitis or rarely intrahepatic abscess.
- Intestinal phase of *A duodenale* cause epigastric pain, inflammatory diarrhea and iron deficiency anemia.

15. Ans. (b) Common variable immunodeficiency *Ref. Robbin's 8/e, p 231*

"Giardiasis is common in blood group A; achlorhydria; cannabis users; chronic pancreatitis; malnutrition; immune defects such a selective IgA deficiency, hypogammaglobulinemia, combined variable immunodeficiency, X-linked and autosomal recessive agammaglobulinemia, and AIDS." *Ref. Harrison, 17/e p 2056*

Infectious agents in different type of immune deficiencies				
Pathogen Type	T-Cell Defect	B-Cell Defect	Granulocyte Defect	Complement Defect
Bacteria	Bacterial sepsis	Streptococci, staphylococci, *Haemophilus*	Staphylococci *Pseudomonas*	Neisserial infections, other pyogenic bacterial infections
Viruses	CMV, EBV, Varicella, chronic infections with respiratory and intestinal viruses	Enteroviral encephalitis		
Fungi and parasites	*Candida, Pneumocystis carinii*	Severe intestinal giardiasis	*Candida, Nocardia Aspergillus*	

16. **Ans. (a, b, c) and (d) Malabsorption commonly seen, Trophozoite form is binucleate pear shaped, Diarrhea is seen and Jejunal wash fluid is diagnostic** *Ref. Paniker 7/e, p 30 - 33; Jawetz 27/e, p 709 - 710*

Already explained

17. **Ans. (c) and (d) Antimonial are useful drugs and Diagnosed by blood smear**
Already explained *Ref. Harrison 19/e, p 1390, 18/e, p 1712; Paniker 7/e, p 56, 59*

18. **Ans. (a) and (c) Leishmania donovani and Leishmania major** *Ref. Harrison 19/e, p 1389 - 1390, 18/e, p 1709*

Leshmania exist in 2 forms:
a. **Amastigote form** : Non-flagellated, occur in macrophage of man as Leishmania donovani (LD) bodies.
b. **Promastigote form** : Flagellated, occur in gut of sandfly and artificial culture.

19. **Ans. (a) *L. braziliensis*** *Ref. Paniker 7/e, p 51*
Already explained

20. **Ans. (b) Trophozoites and cysts are seen in man** *Ref. Paniker 7/e, p 31 - 32; Jawetz 27/e, p 709*

Remember:
- Giardia lamblia lives in the duodenum and upper jejunum and is the only protozoan parasite found in the lumen of human small intestine.

21. **Ans. (a, d, e) Neutropenia, Skin hyperpigmentation, Lymphadenopathy** *Ref. Harrison 18/e, p 1711, 19/e p 1389*

Manifestations of visceral leishmaniasis
- Moderate to high grade fever with chills & rigor
- ***Lymphadenopathy:*** Lymphadenopathy is common in most endemic regions of the world except the Indian subcontinent
- ***Organomegaly:*** Splenomegaly occurs by 2nd week of illness followed by hepatomegally (moderate)
- ***Hyperpigmentation:*** Patient loose weights feel weak and the skin gradually develops dark discoloration due to hyperpigmentation
- ***Hematological:*** Anemia, hypoalbunemia, thrombocytopenia, leukopenia.

22. **Ans. (d) Arthralgia, sore throat and abdominal pain are the most common manifestation**
Ref. Paniker 7/e, p 87 - 89; Harrison 18/e, p 1722 - 1725, 19/e p 1399

Toxoplasma gondii
- It is ***obligate intracellular*** sporozoan.
- It has three forms:
  1. *Trophozoites*
     - It can invade any nucleated cell (i.e. not ***RBC***) and replicate by endodyogeny or internal budding. This rapidly multiplying trophozoite is known as tachyzoites. It can be seen extracellularly also.
     - Crescentic parasites distend the cells which are called as pseudocyst or pseudocolony. It is differentiating from true tissue cyst by its staining property.
     - Stained by Giemsa.
     - It is *non-infective.*
     - It is formed during acute phase.
  2. *Tissue cyst -*
     - It is formed during chronic phase in various organs but persist principally in central nervous system and muscles.

- Cyst contain slowly multiplying rounded parasite called Bradyzoites.
- It is stained by silver stains.

3. *Oocyst* -
   - It develops *only* in intestine of definitive host.
   - It contains two sporocysts with sporozoites inside.

- **Mode of transmission:**
  - Usually by ingestion of either sporulated oocyst from contaminated soil, food, water or bradyzoites from undercooked meat (ingestion of even single cyst can produce infection).
    Also transmited by blood transfusion and organ transplantation and transplacentally.

- **Clinical features:**

-Human toxoplasmosis is zoonosis (Anthropo-Zoonoses). It is of following types:

a. *Toxoplasmosis in immunocompetent person*: Mostly asymptomatic.
   - *MC* manifestation: ***Cervical lymphadenopathy*** which is generalized in 20-30%.
   - Headache, fever, myalgia, splenomegaly often present.
   - Meningoencephalitis, mycocarditis, pneumonitis, chorioretinitis are rare.

b. *Toxoplasmosis in immunocompromised person:*
   - *MC* site is **CNS** (usually brainstem).
   - *MC* symptom: Altered mental status.

c. *Congenital toxoplasmosis*
   - Occurs only when mother gets primary toxoplasmosis infection whether clinical or asymptomatic during pregnancy or <6 months before conception (i.e. no risk if acquired > 6 months before conception).
   - As gestational age is increased, risk of transmission to fetus increased, i.e. max. in 3rd trimester while severity of fetal damage is decreased, i.e. infant is usually asymptomatic if infection transmit in 3rd trimester.
   - It causes:
     - hydrocephalous
     - diffuse cerebral calcification
     - hepatosplenomegaly
     - mental retardation
     - myocarditis
     - lymphadenitis
     - microencephaly
     - myocarditis
     - chorioretinitis
     - multiorgan failure
     - pneumonitis

Summary

- **Asexual** (schizogony) forms of parasite – Trophozoite and tissue cyst
- **Sexual** form (gametogony or sporogony) – Oocyst
- **Definitive** host – Domestic cat and other felines.
  – ***All three forms present.***
- ***Intermediate host*** – Man, mammals and birds; only asexual forms present.
- ***Infective stage*** for man – Oocyst with sporozoites and tissue cyst with bradyzoites.
- Freshly passed oocyst is not infectious (needs development in soil).
- Mature Oocyst containing 8 sporozoites is the infective form.
- Human infection is dead end for the parasite.

23. Ans. (c) Multiple infection of erythrocytes are seen *Ref. Harrison 19/e, p 1369; Paniker 6/e, p 75; 7/e p75*

Blood Smears of Plasmodium				
Features	***P. falciparum***	***P. vivax***	***P. malariae***	***P. ovale***
Features of red cells				
Size	All sizes / normal	Large (young), pale	Small (Old) / Normal	Large (Young)
Shape	Round may be crenated	Round or oval	Round	Round or pear-shaped, fimbriated

Contd...

Contd...

Blood Smears of Plasmodium				
Features	***P. falciparum***	***P. vivax***	***P. malariae***	***P. ovale***
Stippling	Maurer's clefts; Large; red up to 20 Basophilic stippling ±	Schuffner's dots: numerous, small, red	None Occasionally, Zieman's stippling	Schuffner's dots, James dot
Features of Parasite				
Ring (early torphozoite)	Theradlike, multiple infections, double chromatin dots, form accole or appluque	Thicker	Compact	Compact
Mature / Late tropho zoites (amoeboid form)	Absent/occasionally seen	Ameboid, may fill cell	More regular, smaller; Band form	Less ameboid and smaller than those of *P. vivax*;
Schizonts	Absent/occasionally 8-24 merozoites in grape like pattern	12 to 24 merozoites grape like pattern	8 to 12 merozoites, often rosetted around pigment	8 to 12 merozoites irregullarly arranged
Gametocytes	Sausage or crescentic/banana shaped central chromatin (female) or diffuse (male)	Round, fills cell, pigment often central	Round, large coarse pigment	Smaller and oval, but similar to those of *P. vivax*
Diagnostic keys				
	Gametocyte; multiple rings; double chromatin dots; accole forms, heavy infection	Schizont; large RBCs; ameboid forms	Schizont; small RBCs; band forms	Schizont and large pear-shaped fimbriated RBCs

Characteristics of Plasmodium Species infecting Humans				
Characteristic	***P. falciparum***	***P. vivax***	***P. ovale***	***P. malariae***
Incubation period	12 days (shortest)	14 days	14 days	30 days (Longest)
Number of merozoites released per infected hepatocyte	30,000	10,000	15,000	15,000
Duration of erythrocytic cycle (hours)	48 (Malignant tertian malaria)	48 (Benign tertian malaria)	50 (Ovale tertiar malaria)	72 (Quartan malaria)
Red cell preference	Younger cells (but can invade cells of all ages), > 2% of RBC	Red cells up to 14 days old, < 1% of RBC infect	Reticulocytes	Older cells
Morphology	Usually only ring forms; banana shaped gametocytes	Irregularly shaped large rings and trophozoites; enlarged erythrocytes; Schuffner's dots	Infected erythrocytes enlarged and oval with tufted ends; Schuffner's dots	Band or rectangular forms of trophozoites common
Pigment color	Black	Yellow-brown	Dark brown	Brown-black
Ability to cause relapses = Hypnozoites or exo-erythrocytic schizogony	No	Yes	Yes	No

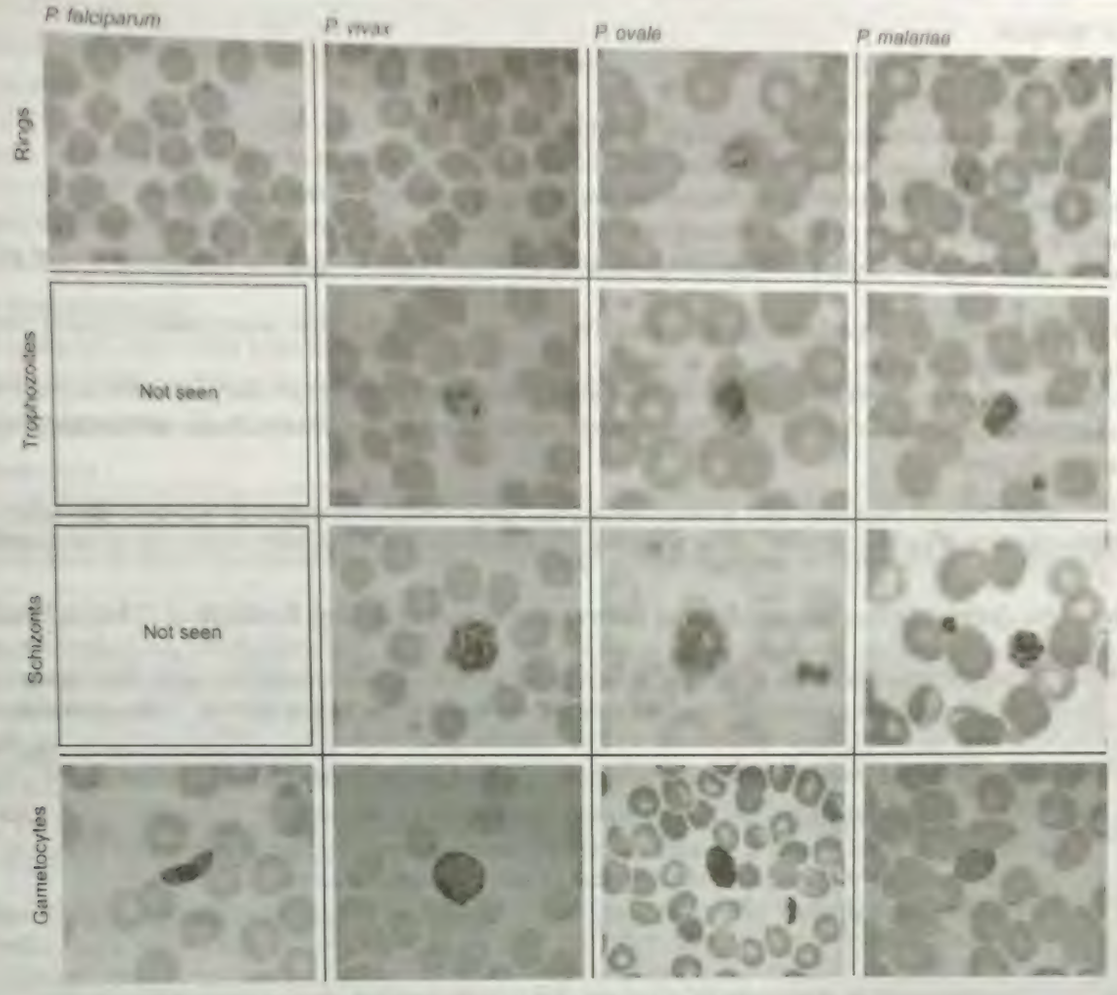
24. Ans. (a) Size of RBC is enlarged *in vivax* infection *Ref. Harrison 19/e, p 1369, 18/e, p 1689; Paniker 7/e, p 72*

Infected RBC's gets enlarged after vivax and ovale infection.

Schuffner dots are seen in P. vivax and P. ovale malaria.

25a. Ans. (a) Plasmodium falciparum *Ref. Paniken 7/e, p 80*

Multiple ring form suggest diagnosis of P. Falciparum



Blood picture in different species of plasmodium

Important features of Falciparum malaria

- Most virulent
- Sporozoites are sickle shaped. Tissue phase consist of only a single cycle of pre-erythrocytic schizogony. No hypnozoites occur
- Ring forms are often seen attached to the margin of red cell forming applique. There can be multiple rings in single RBC
- Late trophozoites and mature schizonts are not ordinarily seen in peripheral blood. The presence of P. falciparum schizonts in peripheral smear indicates a grave prognosis.
- Infected erythrocyte are of normal size and exhibit Maurer's cleft.
- Cause black water fever, pernicious malaria, malignant malaria and septicemic malaria

Why trophozoites and mature schizonts are not seen?
The erythrocytes infected with trophozoites extrudes a strain specific erythrocytes membrane adhesive protein, (PMPI) that mediate erythrocytic attachment to receptors on venular capillary endothelium (RA M-1 in brain, chondroitin sulfate B in placenta).
The infected erythrocyte stick in side and block capillaries and venules. At the same time infected RBC adhere to uninfected RBC to form rosette. This result in sequestration of infected RBC containing mature form of parasite in vital organs like brain, kidney, spleen, etc.

25b. Ans. (b) i.e Due to adherence *Ref. Paniker 7/e, p 71*

The early ring form in the erythrocyte is very delicate and tiny, measuring only a sixth of red cell diameter. Rings are often seen attached along the margin of red cells, the so called *form applique* or *accole*.

25c. Ans. (b) Accole forms are seen *Ref. Harrison 19/e, p 1371, 18/e, p 1689; Paniker 7/e, p 71*

The early ring form in the erythrocyte is very delicate and tiny measuring only a sixth of red cell diameter. Rings are often seen attached along the margin of red cells, the so called *form applique* or *accole*.

26. Ans. (d) Histidine rich protein II (HRPII) *Ref. Harrison 19/e, p 1375 - 1376, 18/e, p 1696; Park 22/e, p 238*

Diagnosis of Malaria

a. *Demonstration of parasite (= Asexual forms)*
   - ***Thin film*** *is fixed and stained*
     - Giemsa (Romanowsky stains) at p H 7.2 is preferred.
     - RBC is examined in tail region for identification of species/type of malaria.
   - ***Thick blood film*** is stained without fixing for searching of *parasites. It is more sensitive.*

b. Rapid, simple, sensitive and specific antibody based diagnostic dip stick (antigen capture assay) or card test that detect *P. falciparum* specific (pf HRP-2) histidine rich protein 2 or lactate dehydrogenase antigen in finger prick blood sample is done.
   - Pf HRP-2 is antibody based test remains positive for several weeks after acute infection so it has disadvantage in high transmission areas.

c. *Malarial fluorescent antibody test is usually becomes positive 2 weeks or more after primary infection so positive test is not necessarily an indication of current infection. It is of greatest value in epidemiological studies and in determining whether a person has had malaria in the past.*

Remember: In severe malaria, poor prognosis is indicated by predominance of more mature P. falciparum parasites (>20% of parasites with visible pigment) in peripheral blood film or presence of phagocytosed malarial pigment in >5% of neutrophils.

27. **Ans. (c) A low iron content in the diet predispose to invasive amoebiasis** *Ref. Park 22/e, p 219-220; Paniker 7/e, p 14 - 18*

Option "a"	:	Trophozoites from acute dysenteric stool, often contain phagocytosed erythrocytes. This features is diagnostic for *E. histolytica* as phagycytosed red cells are not found in the minuta form or in other commensal intestinal amoebae. *...Paniker 7/e, p 21*
Option "b"	:	Amoebiasis is more related to poor sanitation and socioeconomic status than to climate. *....Park 22/e, p 219* More common in adults than in children and in males than in females. *...Paniker 6/e, p 28*
Option "c"	:	Iron in diet is not related to invasive amoebiasis since it spreads by contaminated food and water containing quadrinucleate mature cyst passed in feces of convalescent and carriers.
Option "d"	:	Enzyme electrophoretic mobility analysis have so far identified 7 potentially pathogenic and 11 non- pathogenic zymodemes. *...Park 22/e, p 218*
Remember:		Ordinary residual chlorination of water may not destroy cyst (but kill trophozoites) though super chlorination does. ***Tetraglycine hydroperiodide*** (Iodination) is *best* disinfectant.

28. **Ans. (b) IgM antibodies against *Toxoplasma* in the foetus** *Ref. Harrison 19/e, p 1403, 18/e, p 1726*

Diagnosis of Toxoplasmosis

Diagnosis can be made by appropriate culture, serologic testing and PCR.

i. **Tissue and body fluids:** Although difficult and available only at specialized labs isolation of *T gondii T gondi* from blood or other body fluids can be accomplished after subinoculation of sample. Demonstration of tachyzoites in lymph nodes establishes the diagnosis of acute toxoplasmosis.

ii. **Serology**
   - Commonest method of laboratory diagnosis.
   - Diagnosis of acute infection can be established by detection of simultaneous presence of IgG (by Sabin Feldman dye test, indirect fluorescent antibody test and ELISA) and IgM (by double sandwich IgM ELISA and IgM immunosorbent assay).
   - Presence of IgA also favours diagnosis of acute infection. IgA ELISA is more sensitive than IgM ELISA for detecting congenital infection.

iii. **Molecular diagnosis**
   - Real time PCR for either the B_1 gene or the 529-bp sequence.

Patient type

a. ***Immunocompetent adult or child***
   - Patient with lymphadenopathy only, a positive IgM titre is an indication of acute infection and indication of therapy.

b. ***Immunocompromised host***
   i. Person should be tested for IgG antibody to *T. gondii* soon after diagnosis of HIV infection.
   ii. IgM serum antibody is usually not detectable.

c. *Congenital infection*

i. Antibodies in neonate may be either due to congenital infection (IgG, IgM) or due to passive transfer of antibodies from mother (IgG only).
So detection of IgM specific antibody in fetus is helpful in diagnosing congenital toxoplasmosis since it does not cross placenta.
Harrison writes: ***"Persistence of IgG antibody or positive IgM titer after first week of life – diagnosis of congenital Toxoplasmosis."***

ii. PCR of amniotic fluid to detect B1 gene of the parasite has replaced fetal blood sampling.

d. *Ocular toxoplasmosis*

i. Positive IgG titer with typical eye lesions.
ii. Antibody production is expressed in terms of Goldmann-Witmer coefficient.

29. Ans. (b) IgG antibodies are diagnostic in congenital toxoplasmosis *Ref. Harrison 19/e, p 1403 - 1310; 18/e, p 1727*

Already explained

30. Ans. (b) ***Plasmodium vivax*** *Ref. Harrison 19/e, p 1369, 18/e, p 1688*

Catch clue of answer from question. In this question clue is 'colour of pigment' which are:

- **Black pigment** : *P. falciparum*
- **Brown black** : *P. malariae*
- **Dark brown** : *P. ovale*
- **Yellow brown** : *P. vivax*

Mnemonic: *Learn dark shade to light shade (black to yellow), so species arranged alphabetically.*

31. Ans. (d, e) Laboratory test are useful for making diagnosis, Infection is severe and progressive in immunocompromised host *Ref. Harrison 18/e, p 1722 - 1724, 19/e p 1401*

- Principal source of human Toxoplasma infection remains uncertain but infection is though to occur by oral route and can be attributables to ingestion of either sporulated oocysts from contaminated soil, food or water or bradyzoites from under cooked meat.
- Most common site of brain involvement is brain stem.
- Isolation of *T. gondii* from blood or other body fluids is difficult and available only at specialized laboratories.
- Diagnosis of Toxoplasmosis is based on detection of simultaneous presence of IgG and IgM antibodies to Toxoplasma in serum.
- In immunocompetent individuals toxoplasmosis is usually asymptomatic and self-limited, in contrast immunocompromised individuals experience severe and progressive disease.

32. Ans. (b, c) and (d) Gametocytes, Accole trophozoite and Ring form *Ref. Harrison 17/e, p 1280; 15/e p 1191*

Already explained

33. Ans. (a, b, c) and (d) Thrombocytopenia, DIC, Hemolysis, and Hematemesis *Ref. Harrison 19/e, p 1372, 18/e, p 1692*

Manifestation of severe *falciparum* malaria:

i. ***Cerebral malaria/unarousable coma:***
- Coma is characteristic and omnious feature of *falciparum* malaria.
- Manifest as diffuse symmetric encephalopathy, focal neurologic signs are unusual.

ii. ***Hypoglycemia*** – associated with poor prognosis.
iii. ***Lactic acidosis*** – Plasma conc of HCO^{3-} and lactate are *best* biochemical prognosticators in severe malaria.
iv. ***Noncardiogenic pulmonary edema.***
v. ***Renal impairment.***
vi. ***Hematologic abnormalities:***
- Anemia due to ↑ RBC destruction, removal by spleen and ineffective erythropoiesis.
- Mild thrombocytopenia
- Bleeding with DIC
- Hematemesis due to stress ulceration or acute gastric erosion.

vii. ***Liver dysfunction*** – Associated with poor prognosis.
viii. ***Convulsion, chest infection, catheter induced UTI, septicemia, Salmonella bacteremia.***

34. Ans. (b) and (c) Due to ingestion of oocyst from cat's faeces and Spiramycin given in pregnancy

Ref. Harrison 19/e, p 1404, 18/e, p 1728; Jawetz 27/e, p 684

Treatment of Toxoplasmosis

i. ***Congenital infection:***
- Pyrimethamine and sulfadiazine
- Spiramycin + prednisone

ii. ***Infection in immunocompetent:***
- If only lymphadenopathy : No treatment unless have severe persistent symptoms.
- If ocular toxoplasmosis : Pyrimethamine + sulfadiazine or clindamycin.

iii. ***Infection in pregnancy*** : Spiramycin (rovamycine) is ***DOC.***

iv. ***Infection in Immunocompromised:***
- Prophylaxis for AIDS who are seropositive for *T. gondii* and have CD4 T cell <100/µl : Trimethoprim + sulfamethoxazole/Dapsone + Pyrimethamine.
- Pyrimethamine + Sulfadiazine (preferred but not widely available).

Remember: Sporocyst, sporoblast, sporozoites are formed during sporulation of oocyst in soil not in tissues.

35. Ans. (a) and (b) Caused by Babesis microti and Resides in RBC

Ref. Harrison 17/e, p 1295, 18/e, p 1706; Jawetz 25/e, p 682; Paniker 7/e, p 83

Babesia

- Intraerythrocytic (blood) parasite causing piroplasmosis/Texas fever/Acute hemolytic disease/Babesiosis.
- ***Most*** human infection are caused by *B. microti* and *B. divergens.*
- ***Vector*** Ixodid ticks (*I. dammini* or *I. scapularis, I. ricinus*).
- ***Mode of transmission*** – Tick bite and blood transfusion.
- **Clinical features**
  - Mostly asymptomatic.
  - Characterized by malaise, fever, sweat, depression, myalgia, arthralgia which resembles malaria and rickettsiosis.
  - Most severe illness is seen in immunosuppressed; splenectomized (usually infected by *B. divergens* – may develop jaundice, renal insufficiency) and elderly persons.
  - Rash is absent.
- **Diagnosis**
  - Giemsa stained thick and thin film shows small intraerythrocytic parasites resembling malaria but ***it does not form pigment, schizonts, or gametocytes*** and seen as tetrad forms infrequently.
  - ***'Maltese cross'*** form in RBC *without pigment or gametocyte is diagnostic.*
- **Treatment**

Organisms	Adult/child
B. microti (mild)	Atovaquone + azithromycin
B. divergens and Others	Quinine + Clindamycin + exchange transfusion

36. Ans. (b) and (d) It is one of the common opportunistic infection in AIDS and AFB +ve cyst

Ref. Harrison 19/e, p 1407, 18/e, p 1731; Paniker 7/e, p 94 - 96

Cryptosporidium

- *Cryposporidium* is a Acid fast coccidian intracellular but extracytoplasmic parasite.
- Completes its life cycle in one host (monoxenous).
- **Most human** infection is caused by *C. paroum.*
- **Infective stage:** Oocyst (contain 4 sporozoites) in feces which is *infective immediately* without further development so person to person transmission can occur.
- **Mode of infection:** Acquired from infected animal or human feces or from feces contaminated food or water.
- **Principal site of infection:** Surface of villi of lower small bowel.
- **Clinical features**

In immunocompetent		In immunosuppressed AIDS patient	
•	Self limited watery non-bloody diarrhoea	•	Chronic, persistent and profuse diarrhoea
•	Traveller's diarrhoea can occur	•	Due to involvement of biliary tract papillary stenosis, sclerosing cholangitis or cholecystitis can occur

- **Diagnosis**
  - Fecal examination for small oocyst of 4-5 µm in diameter.
  - Modified acid fast staining for oocyst
  - Fluorescent staining with auramine phenol is advantageous
  - Direct immunofluorescent stains and enzyme immunoassay (for fecal antigen).
  - Biopsy may show *Cryptosporidium* at apical surfaces of intestinal epithelium.
- **Treatment:**
  - No chemotherapy is effective
  - Paromomycin - partially effective in HIV patient
  - Nitrazoxanide - in children
  - Spiramycin or combination therapy with azithromycin is also effective.

37. **Ans. (a) and (b) Splenomegaly and Nephrotic syndrome** *Ref. Harrison 19/e, p 1374 - 1375, 18/e, p 1695*

Chronic complication of malaria are:

i. ***Tropical splenomegaly (Hyperreactive Malarial Splenomegaly).***
   - Produce hypergammaglobulinemia.
   - Normocytic normochromic anemia and splenomegaly.
   - Hepatomegaly with marked elevation of IgM, malarial antibody, hepatic sinusoidal lymphocytosis, peripheral B cell lymphocytosis, formation of cryoglobulins (IgM aggregates and immune complexes), cytotoxic antibodes to suppressor CD-8 cells and increased vulnerability to respiratory and skin infection.

ii. ***Quartan malarial nephropathy of P. malariae causing*** nephrotic syndrome, focal or segmental glomerulonephritis.

iii. ***Burkitt's lymphoma and EBV infection. Due to malaria related immunosuppression.***

38. **Ans. (a) Thick smear to identify parasite** *Ref. Park 22/e p 238; Paniker 7/e, p 79*

Thick film is more sensitive as it concentrates 20-30 layer of blood cells in small area

- Annual blood examination rate, **ABER** = $\frac{\text{Number of slides examined}}{\text{population}} \times 100$
- Incubation period of different malaria is different.

39. **Ans (a)** ***Cryptosporidium*** *Ref. Harrison 17/e, p 1169, 19/e, p 1407; Paniker 7/e, p 96*

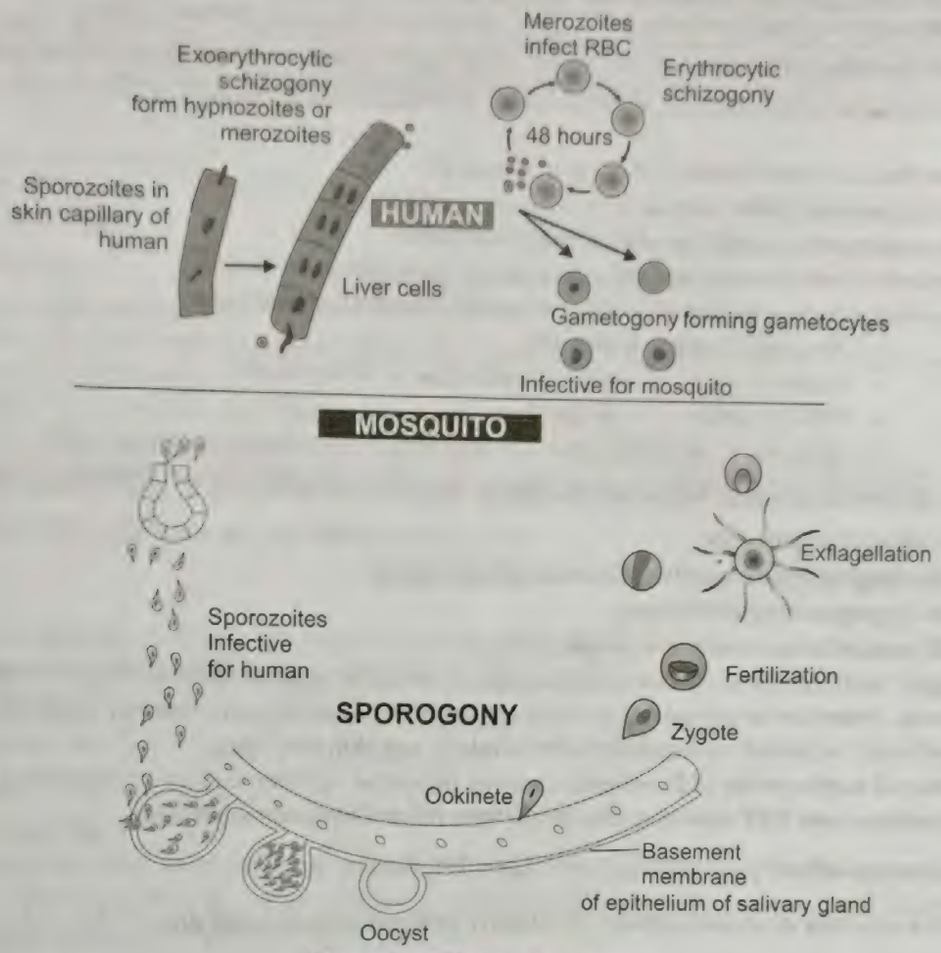
Parasite causing diarrhea on AIDS patient			
Parasite	**Site**	**Mode of infection**	**Oocyst**
Cryptosporidia	Enterocyte in small intestine	Ingestion of oocyst	Blue spherical (≈ 5 mm) bodies in acid fast stain
Microsporidia	Intestine, Muscle, CNS	Spore ingestion	Spores are 2-4 µm in size with polar filaments or tubules
Isospora belli	Epithelial cells of small intestine	Ingestion of mature oocyst	Oval or flask shaped, 25 µm × 15 µm with two sporocyst and four sporozoites. Also acid fast

Remember: Coccidian parasites are *Isospora*, *Toxoplasma* and *Cryptosporidia*
Blastocytis hominis is round cell of 6-40 µm containing large membrane bound central body with nuclei in cytoplasm peripherally.

40. **Ans. (a) Gametocyte** *Ref: Paniker Parasitology 7/e, p 66, 67*

- Infective forms for human is sporozoites in saliva of mosquito
- Infective forms for mosquito is gametocytes in human blood. At least 12 gametocytes per cubic mm of blood must be present to infect mosquito.
  - Gametocytes are maximum in number during the early stages of infections (may exceed 1000 per cubic mm of blood).
  - Nonmotile zygote converted into motile ookinete in about 18 - 24 hours.

- Human reservoir is one who harbours the sexual forms (gametocytes) of the parasite which is infective for mosquito.



Life cycle of Plasmodium vivax

41. Ans. (d) Detects histidine rich protein 1 *Ref. Harrison 19/e, p 1376*

Antigen capture assay detects histidine rich protein II not HRP-I

42. Ans. (c) IgG is diagnostic *Ref. Harrison 17/e, p 1309-1310, 18/e, p 1727*
Already explained

43(a). Ans. (c) Isospora *Ref: Harrision 19/e, p 1407, 18/e, p 1732; Paniker 7/e 93*

Isospora belli (Cytoisosporiasis)

- Coccidian parasite
- ***Site of infection*** – Epithelial cells of small intestine where schizogony (sickle shaped merozoites are formed) as well as sporogony occur.
- ***Infective stage***–Mature oocyst. (forms outside the human body) containing two sporocyst with four sporozoites each.
- ***Mode of transmission***–Ingestion of mature oocyst in food or drink.

Clinical features

- Usually asymptomatic or abdominal discomfort, mild fever and diarrhea in self-limited manner
- Protracted diarrhea can occur in immunocompromised person especially in HIV patient.
  – Diagnosis–demonstration of acid fast oocyst in fecal smears.

Treatment

- Cotrimoxazole is effective
- It is easy to treat but relapses are common in comparison to diarrhea of cryptosporidium

Remember: Oocyst of cryptosporidium is small, hard and is fully mature on release while that of isoopora oocyst is large, transparent, thin walled, flask shaped and mature outside the human body.

43(b). **Ans. (b) P. falciparum** *Ref. Harrison 19/e, p 1369*

This is a thin smear representing ring forms of P. falciparum.

For details of blood picture see image platelets.

44. **Ans. (b, c) and (d) *Naegleria, Acanthamoeba* and *Balamuthia*** *Ref. Harrison 17/e, p 2632*

ENCEPHALITIS (Infection of brain parenchyma)			
Viral	**Bacterial**	**Fungal**	**Parasitic**
– HSV1 ***(MC)***	– *Listeria*	– *Cryptococcus*	– *Naegleria*
– VZV	– *Mycoplasma*	– *Mucor*	– *Acanthamoeba*
– Enterovirus	– *Leptospira*		– *Balamuthia*
			– *Baylisascaris procyonis*
			– *Toxoplasma*

45. **Ans. (b) and (e) It is a protozoa and It is associated with diarrhoea in HIV patients** *Ref. Harrison 19/e, p 1408, 18/e, p 1732*

- **Microsporadia** are gram-positive, obligate intracellular, spore forming protozoa that cause disease in humans especially as opportunistic pathogens in **AIDS.** It reside in cytoplasm of enteric cells.
- Main species causing infection in humans is Enterocytozoon bieneusi.
- Also seen in extraintestinal locations like muscles, eye (conjunctivitis) and liver (hepatitis) in comparison of isospora or cryptosporidium.
- In **AIDS** patient microsporidium cause diarrhoea.
- **Diagnosis** is made by demonstration of spore in smear of faeces or duodenal aspirate by modified trichome or chromotrope 2R based staining or Uveitx 2B or calcofluor fluoroscent staining.

46. **Ans. (b) and (d) Naegleria and *Angiostrongylus cantonensis*** *Ref. Jawetz 23/e, p 688*

Already explained

47. **Ans. (b) i.e. Opisthorchis** *Ref. Internet reference*

Organism	Size	
	Cyst	Trophozoite
E. histolytica	12-15 μm	15-30 μm
E. Coli	10-35 μm	20-50 μm
Giardia	9-12 μm	12-15 μm
B. coli	30-200 μm	30-50 μm
C. Cryptosporidium parvum	4-5 μm	3-5 μm
Isospora belli	25-30 μm	

Opisthorchis: Trematode fluke resembling C. sinesis.

Chapter Review

1. **Band shaped trophozoites are seen in:** [AI 90]
   a. *P. ovale* b. *P. vivax*
   c. *P. falciparum* d. *P. malariae*
   [Ref. Paniker 7/e, p 75]

2. **Diagnostic test for amoebic hepatitis is:** [AI 90]
   a. Indirect hemagglutination test
   b. Isolation from pus
   c. Isolation from wall of cavity
   d. Cyst in stools *[Ref. Harrison 17/e, p 1277]*

3. **The normal habitat of giardia is:** [AIIMS 90]
   a. Duodenum and jejunum
   b. Stomach
   c. Caecum
   d. Ileum *[Ref. Paniker 7/e, p 31]*

4. **Amoebic liver abscess can be diagnosed by demonstrating:** [AI 91]
   a. Cysts in the sterile pus
   b. Trophozoites in the pus
   c. Cysts in the intestine
   d. Trophozoites in the faces *[Ref. Paniker 7/e, p 22]*

5. **The main reservoir for *Entamoeba histolytica* is:**
   a. Man b. Dirty water [AI 91]
   c. Soil d. Ponds
   [Ref. Park 22/e, p 218]

6. **All are true about *Entamoeba histolytica* except:**
   a. Cyst are 8 nucleated [AI 91]
   b. Cyst are 4 nucleated
   c. Trophozoites colonise in the colon
   d. The chromatid bodies are our stained by iodides
   [Ref. Paniker 7/e, p 16]

   Early cyst contains a single nucleus whereas mature cyst has four nucleus or is quadridentate.

7. **The following is true of Giardiasis except:** [AI 92]
   a. Complement fixation test is diagnostic
   b. Stools contain only cysts
   c. Habitat is colon
   d. Trophozoite and cysts are found in duodenum
   [Ref. Paniker 7/e, p 33]

8. **The cystic form of all are seen in man except:**
   a. *E. histolytica* b. *Girdia* [AI 92]
   c. *Trichomonas* d. *Toxoplasma*
   [Ref. Paniker 7/e, p 35]

   T. vaginalis does not form cyst and the trophozoite itself is the infective form.

9. **Which is true of malaria?** [AI 93]
   a. Rods forms are seen in *P. malariae*
   b. RBC size is more in *P. vivax*
   c. Relapse seen in *P. falciparum*
   d. Male and female mosquito transmit disease
   [Ref. Harrison 19/e, p 1689 (Table 210.1)]

10. **Which is true of torphozoites of *E. histolytica*?** [AI 93]
    a. Has eccentric karyosome
    b. Nuclear membrane without chromatin
    c. Shows erythrophagocytosis
    d. Presence of bacteria inside cell *[Ref. Paniker 7/e, p 15]*

11. **Primary amoebic meningoencephalitis is caused by:** [Kar 00]
    a. *Naegleria fowleri* b. *Entamoeba hystolitica*
    c. *Endolimax nana* d. *Dientamoeba fragilis*
    [Ref. Paniker 7/e, p 25]

12. **Malarial parasites are easily detected if blood films are taken and examined:** [Kar 01]
    a. When the patient is febrile
    b. When the patient is having chills
    c. One hour after the height of the paroxysm
    d. When the patient is afebrile *[Ref. Nelson, p 1050]*

13. **Spot the incorrect statement regarding *Entamoeba histolytica*:** [Kolkata 02]
    a. Cysts are necessary for the transmission of infection from one host to another
    b. Cysts are necessary for the transmission of infection from one host to another
    c. A cyst may be transferred into a trophozoite to cause invasion in the same
    d. Cysts are found in the submucosa of the lower intestinal wall *[Ref. Paniker 7/e, p 16]*

14. **In India kala azar is non-zoonotic disease with man as the sole reservoir though it has many animal reservoir e.g. dogs, jackals, etc. The most important reservoir of Leishmaniasis in India is:** [JIPMER 02]
    a. Dogs
    b. Rodents
    c. Acute visceral leishmaniasis
    d. Case of postkala azar dermal leishmaniasis
    [Ref. Park 22/e, p 278]

Answers 1. d. P. malariae 2. a. Indirect ... 3. a. Duodenum ... 4. b. Trophozoites ... 5. a. Man
6. a. Cyst are 8 ... 7. a. Complement ... 8. c. Trichomonas 9. b. RBC size is ... 10. c. Shows ...
11. a. Naegleria ... 12. c. One hour ... 13. c. A cyst may ... 14. c. Acute ...

15. The infective form of malarial parasite in the vertebrate host is: **[Kar 03]**
a. Sporozoite b. Merozoite
c. Schizont d. Gametocyte
[Ref. Paniker 7/e, p 66]

16. In malaria the Shuffner's dots are due to: **[SGPGI 03]**
a. The disintegrated parts of the parasite
b. The intracellular space not occupied by the parasite
c. The pigment released from breakdown of hemoglobin
d. The gametocytes *[Ref. Paniker 7/e, p 67]*

17. In *falciparum* malaria all of the following are seen except: **[SGPGI 03]**
a. Crescent shaped gametes
b. Maurer's dots
c. Schizont
d. Merozoites *[Ref. Harrison 15/e, 1191]*

18. Babesiosis is transmitted: **[Kar 04]**
a. Tick b. Mites
c. Flea d. Mosquito
[Ref. Paniker 7/e, p 84]

19. Complication in malaria are commonly with: **[Kar 04]**
a. *Plasmodium ovale*
b. *Plasmodium vivax*
c. *Plasmodium falciparum*
d. *Plasmodium malariae* *[Ref. Harrison, 18/e, p 1692; Park 22/e p 237]*

20. Which of the following parasite passes through three hosts? **[Kolkata 04]**
a. *Fasciola hepatica*
b. *Fasciola buski*
c. *Schistosoma haematobolium*
d. *Clonorchis sinensis* *[Ref. Paniker 7/e, p 148]*

21. *P. vivax* attacks: **[Jharkhand 04]**
a. Reticulocytes b. Young RBC
c. Old RBC d. Dead RBC
[Ref. Panikar 7/e, p 69]

22. *Entamoeba* which is not found in gut: **[MP 04]**
a. *E. coli* b. *E. histolytica*
c. *E. gingivalis* d. *E. nana*
[Ref. Paniker 7/e, p 24]

23. Largest intestinal protozoa is: **[DNB 04]**
a. *Entamoeba coli* b. *Balantidium coli*
c. *Giardia lamblia* d. *Toxoplasma gondii*
[Ref. Panikar 7/e, p 107]

B. coli is the only ciliate protozoan parasite of human. It is the largest protozoan parasite of human.

24. Route of transmission of *Toxoplasma*: **[DNB 04]**
a. Blood b. Faeces
c. Urine d. None
[Ref. Harrison 18/e, p 1723]

25. Most fatal amoebic encephalitis is caused by:
a. *Entamoeba histolytica* **[Bihar 05]**
b. *Naegleria*
c. *Entamoeba dispar*
d. *Acanthamoea* *[Ref. Harrison 18/e, p 1686]*

26. Nephrotic syndrome is caused by:
a. *P. falciparum* b. *P. ovale* **[Bihar 05]**
c. *P. vivax* d. *P. malariae*
[Ref. www.pubmedcentral.nih.]

27. In malaria reservoir, parasite remains as:
a. Merozoite b. Sporozoite **[Bihar 06]**
c. Trophozoite d. None
[Ref. Chaterjee Parasitology, p 113; Ghai 6/e, p 241]

28. Amoebae not found in human intestine: **[PGI 97]**
a. *E. histolytica* b. *E. coli*
c. *E. nana* d. *E. gingivalis*
[Ref. Paniker 7/e, p 24]

- Entamoeba gingivalis is present in the mouth, being found in large numbers when oral hygiene is poor.
- It has no cystic stage so transmit by kissing, airborne droplet and by fomites.

29. Oocyst of toxoplasma is found in: **[AIIMS 97]**
a. Cat b. Dog
c. Mosquito d. Cow *[Ref. Paniker 7/e 89]*

30. Stage of *falciparum* not seen in PBS is: **[PGI 99]**
a. Schizont b. Gametocyte
c. Ring form d. Double ring
[Ref. Harrison 15/e 1191]

31. Tachyzoites are seen in: **[PGI 97]**
a. *Toxoplasma* b. *Toxocara*
c. Pulm eosinophilia d. *Ascaris* *[Ref. Paniker 7/e 88]*

Rapidly proliferating trophozoites in acute inferctions are called tachyzoiles.

32. In *Plasmodium falciparum* following are seen in blood except: **[PGI 97]**
a. Schizonts b. Mature trophozoite
c. Mature gametocytes d. None
[Ref. Paniker 7/e, p 71]

Subsequent stages of a sexual cycle-late trophozoite, early and mature schizoinls are not ordinarily seen in peripheral blood in cases of falciparum malaria except in very sever or perncious malaria.

Answers 15. a. Sporozoite 16. c. The pigment ... 17. c. Schizont 18. a Tick 19. c. Plasmodium ...
20. d. Clonorchis 21. b. Young RBC 22. c. E. gingivalis 23. b. Balantidium 24. a. Blood
25. b and d. Naegleria... 26. d. P. malariae 27. d. None 28. d E. gingi...
29. a. Cat 30. a. Schizont 31. a. Toxoplasma 32. a and b. Schizonts...

33. Cerebral malaria is caused by *Plasmodium:*
a. *Falciparum* b. *Ovale* **[PGI 97]**
c. *Malaria* d. *Vivax*
[Ref. Harrison 17/e 1284]

34. Charcot leyden crystals are derived from: [DNB 2007]
a. Macrophages b. Eosinophils
c. Basophils d. Neutrophils
[Ref. Harrison 18/e481]

35. Charcot Leyden crystal in stool in seen in: [DNB 2012]
a. Amoedbic dysentery b. bacillary dysentery
c. Shigella d. bacillus cereus
[Ref. Paniker 7/e, p 20]

36. Compound used for fixation of protozoa found in stool is: [DNB 2012]
a. Phenol b. Hypochlorite
c. Formalin d. Alcohol
[Ref. Paniker 6/e, p 233]

37. Cryptosporidium cyst identified by which stain in stool sample? [DNB 2010]
a. PAS b. H and E
c. Giemsa d. Acid fast stain
[Ref. Harrison 18/e, p 1732; Paniker 7/e, p 96]

38. Investigation of choice for amoebiasis is: [DNB 2012]
a. ELISA b. Colonoscopy
c. Microscopy d. Microscopy + ELISA
[Ref Harrison 18/e, p 1685]

39. True about cryptosporidium are all EXCEP: [DNB 2012]
a. Oocyst chorine resistant
b. Acid fast spores
c. Oocyst >100 micro meters
d. Enzyme immune assay done
[Ref. Paniker 7/e, p 96]

40. MC anopheles vector for transmission of malaria in urban area is: [DNB 2012]
a. An stephensi
b. A. gambiae
c. Both
d. None
[Ref. Park 22/e p 236]

- In India An. culicifacies acts as malaria vector in rural areas and An. steptosis in urban areas

41. Infective form for mosquito in plasmodium falciparum: [DNB 2013]
a. Merozoites
b. Sporozoites
c. Gametocytes
d. Trophozoites
[Ref. Harrison 18/e p 1688]

42. Toxoplasmosis is spread by: [DNB 2013]
a. Dog b. Cat
c. Human d. None
[Ref. Harrison 18/e p 1722]

43. Which of the following is true about mature cysts of *E. histolytica*? [AI 96]
a. Endoplasm and ectoplasm are clearly defined
b. Eight chromatid bodies
c. Shows chromatid bodies and glycogen mass
d. Nuclear structure retains characteristics of trophozoite

Answers 33. a. Falciparum 34. a. Macrophages 35. a. Amoebic dysentery 36. d. Alcohol 37. d. Acid fast stain
38. d. Microscopy... 39. c. Oocyst... 40. a. An stephensi 41. c. Gameto... 42. b. Cat
43. d. Nuclear...

NEET Pattern Questions

1. **The most common cause of malaria in India:**
   a. P. vivax
   b. P. falciparum
   c. P. malariae
   d. P. ovale *[Ref. Park 22/e, p 234]*

In India, 50% of infections are reported to be due to P. falciparum; 4-8% due to mixed infection and rest due to P. vivax

2. **True about P. falciparum includes, all except:**
   a. Duration of erythrocytic cycle is 48 hrs
   b. Exo-erythrocytic phase is absent
   c. Parasitic burden can be estimated by peripheral parasitemia
   d. Cause rosette promotion *[Ref. Harrison 18/e, p 1691]*

RBC infected with P. falciparum adhere to venous wall and other RBC's (to form rosette). This results in sequestration of infected RBC's in vital organs. As a consiquence the level of peripheral parasitemia under estimates the true number of parasites.

3. **Which type of malaria is associated with renal failure?**
   a. Falciparum
   b. Vivax
   c. Malariae
   d. Ovale *[Ref. Paniker 7/e, p 77]*

Renal failure occurs in malignant tertian malaria (particularly in black water fever) which is caused by P. falciparum.

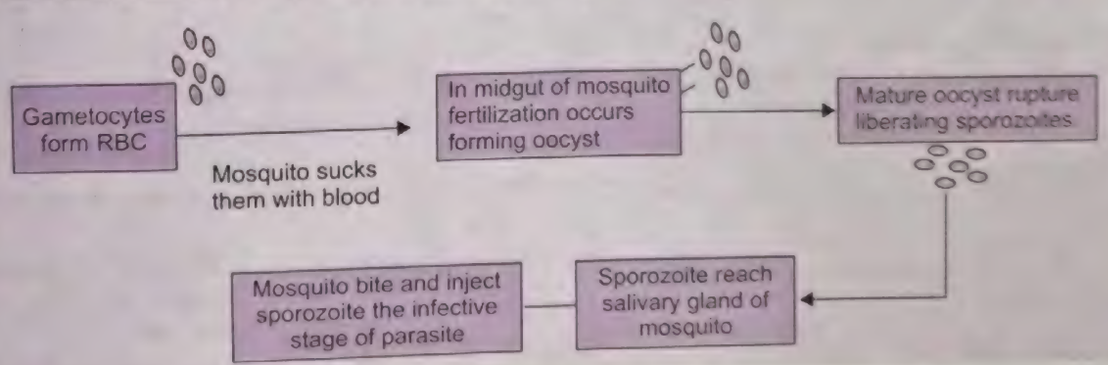
4. **Causative agent of malaria:**
   a. Protozoa
   b. Mosquito
   c. Bacteria
   d. Virus *[Ref. Paniker 7/e, p 64]*

Mosquito is the vector where as plasmodium is the causative agent which is a protozoa.

5. **Malaria causing nephrotic syndrome:**
   a. P. vivax
   b. P falciparum
   c. P. malariae
   d. P. ovale

6. **In malaria, sexual cycle is:**
   a. Sporozoite to gametocytes
   b. Gametocytes to sporozoite
   c. Occurs in human
   d. Responsible for relapse *[Ref. Paniker 7/e, p 65]*

Sexual cycle of plasmodium occurs in mosquitos and is called as sporogony.



7. **Malaria parasite was discovered by:**
   a. Ronald Ross
   b. Paul Muller
   c. Laveran
   d. Pampania *[Ref. Paniker 7/e, p 64]*

Laveran discovered the malarial parasite in RBC of a patient in Algeria. Whereas Ronald Rose established its mode of transmission in Secundrabad.

8. **Malaria carriers contain:**
   a. Trophozoite
   b. Gametocytes
   c. Merozoites
   d. Trophozoites *[Ref. Park 22/e, p 233]*

Answers 1. b. P. falciparum 2. c. Parasitic burden can... 3. a. Falciparum 4. a. Protozoa
5. b. P. falciparum 6. b. Gametocytes to sporozoite 7. c. Laveran 8. b. Gametocytes

9. The scientist who discovered the transmission of malaria by anopheline mosquito:
a. Laveran
b. Paul Muller
c. Ronald Ross
d. Pampana *[Ref. Paniker 7/e p 64]*

10. Protozoa associated with megaesophagus:
a. Trypanosome
b. Ameba
c. Giardia
d. Gnathostoma *[Ref. Paniker 7/e, p 47]*

In chromic phase T. cruzi produces inflammatory response, cellular destruction and fibrosis of muscles and nerves that control tone of hollow organs like heart, oesophagus, colon etc. Thus it can lead to cardiac myopathy and mega-oesophagus and megacolon

11. Largest intestinal protozoa is:
a. E. coli
b. Balantidium coli
c. Giardia
d. T. gondii *[Ref. Paniker 7/e, p 107]*

Balantidium coli is the largest protozoan parasite. It resides in large intestine. It is the only ciliate protozoan parasite of human .

12. Definite host of toxoplasma:
a. Man b. Dog
c. Cat d. Rat
[Ref. Harrison 18/e, p 1722]

Giardia lives in small intestine.

13. Toxoplasma in children causes:
a. Chorioretinitis b. Conjunctivities
c. Keratitis d. Papillitis
[Ref. Harrison 18/e, p 1722-1725]

14. True about Giardiasis:
a. Only cyst is infective
b. Reside in caecum
c. Only man to man transmission
d. Exist in one phase
[Ref. Paniker 7/e, p 31]

15. Most common clinical feature of toxoplasmosis in an immunocompetent adult:
a. Encephalitis
b. Lymphadenopathy
c. Chorioretinitis
d. Glaucoma
[Ref. Paniker 7/e, p 91]

Commonest manifestation of toxoplasmosis:
Acquired: Lymphadenopathy
Congenital: Chorioretinitis, cerebral calcifications
In immunocompromised: Encephalitis, altered mental state.

16. Band form of P malariae is:
a. Schizont stage b. Trophozoite stage
c. Merozoite stage d. Gametocyte stage
[Ref. Paniker 7/e, p 73]

The old trophozoites are seen stretched across the erythrocyte as a broad band. These band forms are unique feature of P.malariae.

17. Which of the following is true about P. falciparum?
a. James dots are seen
b. Accole forms are seen
c. Relapses are frequent
d. Longest incubation period
[Ref. Paniker 7/e, p 71]

18. Schizoints are not seen in peripheral blood of which malarial parasites?
a. P vivax b. P falciparum
c. P ovale d. P malariae
[Ref. Paniker 7/e, p 67]

In P.falciparum, erythrocytic schizogony takes place inside the capillaries and vascular led of internal organs. So, in P.falciparum infections schizonts and merozoites are usually not seen in peripheral blood.

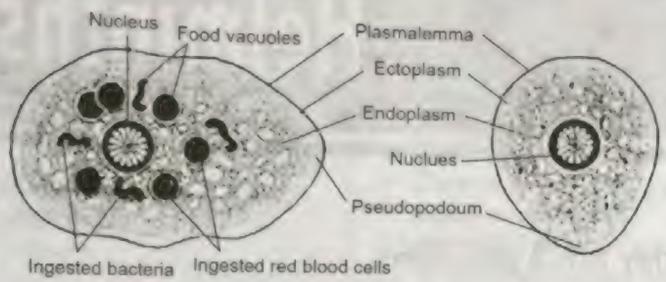
19. Mucocutaneous leishmaniasis is caused by:
a. L-braziliensis b. L. tropica
c. L. donovani d. L. orientalis
[Ref. Paniker 7/e, p 50]

Muco-cutoneous leishmania is caused by L.braziliensis and L. mexicana complex.

20. Invasive amoebiasis can be best diagnosed by:
a. ELISA
b. Countercurrent immunoelectrophoresis
c. Indirect hemaglutination test.
d. Complement fixation text
[Ref. Paniker 7/e, p 22]

21. Trophozoite of Entamoeba histolytica:
a. Has eccentric Karyosome
b. Shows erythrocyte in cytosol
c. Central nucleus
d. Non motile
[Ref. Paniker 7/e, p 15]

Answers 9. **c.** Ronald Ross 10. **a.** Trypanosome 11. **b.** Balantidium coli 12. **c.** Cat 13. **a.** Chorioretinitis
14. **a.** Only cyst... 15. **a.** Encephalitis 16. **b.** Trophozoite... 17. **b.** Accole forms... 18. **b.** P falciparum
19. **a.** L-braziliensis 20. **c.** Indirect... 21. **b.** Shows erythrocyte



22. A patient presents with fever. Peripheral smear shows band across the erythrocytes. Diagnosis is:
a. P Falciparum b. P vivax
c. P ovale d. P malariae
[Ref. Paniker 7/e, p 73]

23. The cystic form of all are seen in man except:
a. E. histolytica b. Giardia
c. Trichomonas d. Toxoplasma
[Ref. Paniker 7/e, p 91]

24. Parasites for which modified ZN stain is used:
a. Isospora b. Microsporidia
c. Plasmodium d. Echinococcus
[Ref. Paniker 7/e, p 231]

Modified Ziehn-Neelsen stain
- Oocyst of cryptosporidium and isospora in fecal specimens may be difficult to detect without special staining. Application of heat to the carbol fuchsin assists in staining.

25. Giardiasis true is all except:
a. Diarrhea with steatosis
b. Bloody diarrhea
c. Metronidazole is the drug of choice
d. Absent fever *[Ref. Paniker 7/e, p 33]*

26. A patient presents with headache, high fever an meningismus. Within 3 days he becomes unconscious. Most probable causative agent:
a. Naegleria fowleri
b. Acanthamoeba castellani
c. Entamoeba histolytica
d. Trypanosoma cruzi *[Ref. Paniker 7/e, p 29]*

27. The normal habitat of giardia is:
a. Duodenum and jejunum
b. Stomach
c. Caecum
d. Ileum *[Ref. Paniker 7/e, p 30]*

28. Kala azar is:
a. Mucocutaneous leishmaniasis
b. Cutaneous leishmaniasis
c. Oriental sore
d. Visceral leishmaniasis *[Ref. Paniker 7/e, p 54]*

29. Amoebiasis is not transmitted by:
a. Feco-oral route
b. Sexual transmission
c. Blood and blood products
d. Vector transmission *[Ref. Harrison, 19/e, p 1362]*

30. Trypanosoma cruzi is transmitted by:
a. Tse tse fly
b. Reduviid bug
c. Culex mosquito
d. Sand fly *[Ref. Paniker 8/e, p 42]*

31. Amastigote form of which parasite is found in human?
a. Trypanosoma cruzi
b. Trypanosoma brucei
c. Trypanosoma gambiense
d. Trypanosoma rhodesiense *[Ref. Paniker 7/e, p 39]*

Amastigote form is found in humans in Trypanosoma cruzi and leishmania

32. Most common clinical feature of toxoplasmosis in an immunocompetent adult:
a. Encephalitis b. Lymphadenopathy
c. Chorioretinitis d. Glaucoma
[Ref. Paniker 8/e, p 90]

33. The pathogenecity of Entamoeba histolytica is indicated by:
a. Isoenzyme pattern b. Size
c. Nuclear pattern d. ELISA test
[Ref. Paniker 7/e, p 18]

34. Duodenal aspirate is used in diagnosis of:
a. E histolytica b. Giardia lamblia
c. Taenia solium d. Leishmania
[Ref. Paniker 7/e, p 33]

35. Recrudescences are commonly seen in which malaria:
a. P vivax b. P ovale
c. P malariae d. P falciparum
[Ref. Paniker 7/e, p 78]

Recrudescence is commonest in falciparum malaria

36. Cerebral amaebiasis is not caused by:
a. Naegleria b. Acanthamoeba
c. Dientamoeba d. Balamuthia
[Ref. Paniker 7/e, p 24]

Cerebral amaebiasis
- Acanthamoeba
- Naegleria Fowleri
- Balamuthia mandrillaris

Answers 22. d. P malariae 23. d. Trichomonas 24. a. Isospora 25. b. Bloody diarrhea 26. a. Naegleria fowleri
27. a. Duodenum... 28. d. Visceral leishm... 29. d. Vector trans... 30. a. Tse tse fly 31. a. Trypanosoma cruzi
32. b. Lymphadenopathy 33. a. Isoenzyme... 34. b. Giardia lamblia 35. d. P falciparum 36. c. Dientamoeba

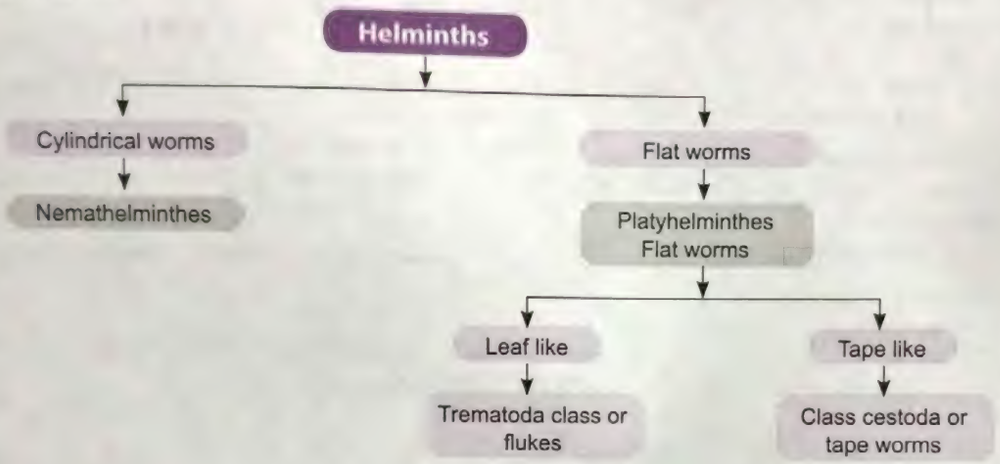
CHAPTER

35

Helminths

Bilaterally symmetrical metazoa belonging to phylum-scolecida. They have 3 germ layers (triploblastic metazoa)

Classification



I
Trematode: Fasciola, Clonorchis.

TREMATODES

- Flat, fleshy, unsegmented worms
- Alimentary canal present, but incomplete without an anus
- Possess suckers, but hooks are lacking
- Sexes may be separate (as in schistosomes) or hermaphroditic
- Important members are:
  - *Schistosomes hematobium:* Lives in vesical and pelvic plexuses of veins
  - *Fasciola:* Lives in biliary tract
  - *Echinostoma:* Lives in intestine
  - *Paragonimus:* Lives in lungs
  - *Clonorchis:* Lives in the biliary tract
  - *Heterophyes:* Smallest parasitic trematode

I
S. hematobium: Endemic humaturia
S. japonium: Katayama fever

CESTODES

- Segmented tape like worms with variable size (few mm to several meters)
- Lacks alimentary canal
- Possess suckers and in some members hooks too
- Sexually hermaphrodites
- Important members are:
  - Diphyllobothrium latum: Lives in human intestine. (=Fish tape worm)
  - Taenia saginata (beef tapeworm): Lives in Jejunum, found in beef eaters
  - Taenia solium (Pork tapeworm): Lives in jejunum. Found in pork eaters mainly
  - Echinococcus granulosus: Causative agent of hyadatid disease. Dogs are the definitive host
  - Hymenolepsis nana (=Dwarf tapeworm): Smallest and the most common tape worm found in intestine.

I
Cestodes: Taenia, Echinococcus

I
Longest Cestode infecting man: Diphyllobothrium latum
Smallest cestode infecting man: hymenolepsis nana

I

Nematodes:
Ascaris: Largest
Parthenogenetic: Strongyloides.
Lives in appendix: Trichuris.

NEMATODES

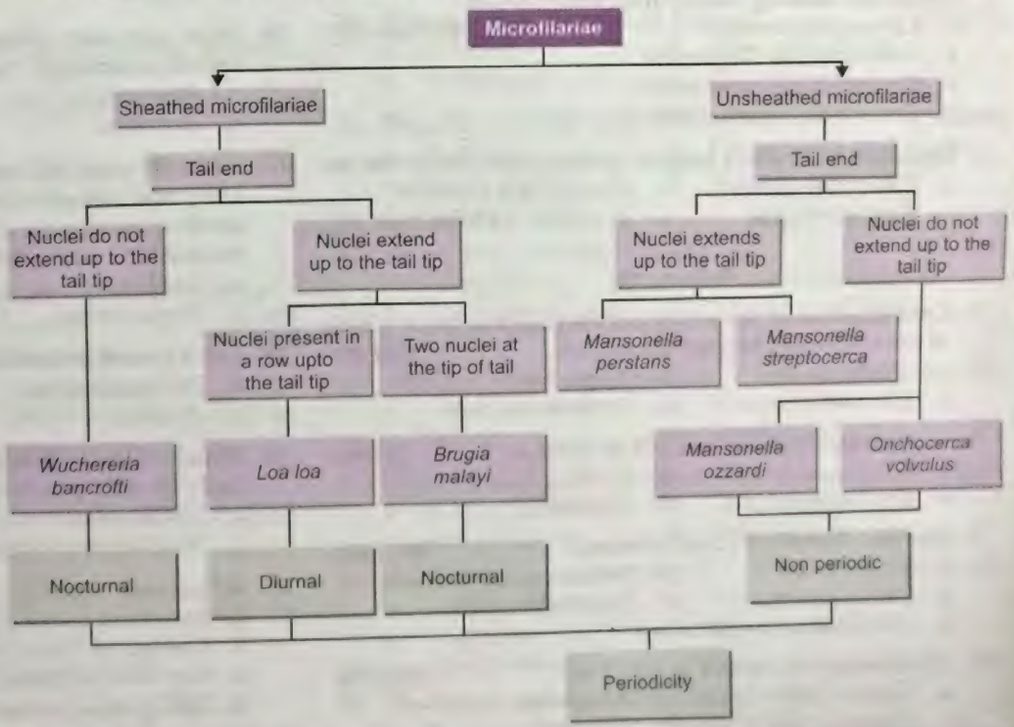
- Resemble common earthworms
- Elongated cylindrical unsegmented worms with tapering ends
- Body is covered with tough cuticle
- Possess alimentary canal
- Sexes are separate
- *Important members are:*
  - Ascaris: Largest nematode parasite-Lives in small intestine of infected person
  - Enterobius: Human pinworm, thread worm on seatworm. Common in developed countries-Adult worm lives in caecum.
  - Ancylostoma duodenal: Lives mostly in Jejunum and only rarely in duodenum, Human are the natural host
  - Strongyloides: Lives in duodenum and upper jejunum. Parthenogenetic in character (i.e. can produce offspring without fertilization)
  - Trichuris trichiura: Likes in caecum and appendix. It is not a blood sucker and there is prominence of mechanical symptoms
  - Trichinella spiralis: Lives in muscle.

FILARIAL WORMS

I

Monsonella ozzardi is virtually nonpathogenic filariasis.

- Nematodes belonging to the super family Filarioidea
- Worms resides in:
  - Subcutaneous tissue (subcutaneous filariasis) : Loaloa, Onchocerca volvulus, Mansonella streptocerea
  - Lymphatic filariasis: Wuchereria boncrofti, Brugia malayi, Brugia timorii.
  - Serous cavity (serous cavity filariasis): Monsonella
  - Female worms are viviparous and give birth to larvae known as microfilariae.



Note:

Filarial worm	Disease
– Onchocerca volvulus	River blindness or onchocerciasis
– Loa loa	Fugitive swellings or calabar swellings
– Wuchereria bancrofti	Filariasis

Multiple Choice Questions

Flukes

1. **Pancreatic Ca is caused by:** [AI 01]
   a. *Fasciola* b. *Clonorchis*
   c. *Paragonimus* d. None

2. **Megaloblastic anemia is caused by:** [AIIMS 95]
   a. *Diphyllobothrium latum*
   b. *Schistosoma hematobium*
   c. *Echinococcus granulosus*
   d. *Taenia solium*

3. **Cholangiocarcinoma is caused by:** [PGI 02]
   a. *Fasciola* infestation
   b. *Clonorchis* infestation
   c. *Paragonimus* infestation
   d. Ascaris infestation
   e. None of these

4. **A traveller present with conjugated hyper-bilirubinemia and on investigations a egg was found in his biliary tract. Likely organism:** [AIIMS May 09]
   a. *Clonorchis sinensis* b. *Fasciola buski*
   c. *Gnathostomata* d. *Ascaris*

5. **Organism causing biliary tract obstruction:**
   a. Clonorchis sinensis [AIIMS Nov 10]
   b. Ankylostoma duodenale
   c. Strongyloides stericoralis
   d. Enterobius vermicularis

6. **Ingestion of raw fish leads to gallbladder cancer due to:**
   a. *Clonorchis sinensis* b. *Hymenolepis diminuta*
   c. *Angiostrongylus* d. *D. latum* [AIIMS Nov 10]

Tapeworms

7. **Consumption of uncooked pork is likely to cause which of the following helminthic disease:** [AI 01]
   a. *Taenia saginata* b. *Taenia solium*
   c. Hydatid cyst d. *Trichuris trichiura*

8. **Commonest parasite of CNS in India is:** [AI 99]
   a. Schistosomiasis b. Cysticercosis
   c. *Trichinella spiralis* d. Hydatid cyst

9. **Hydatid disease of liver is caused:** [PGI 01]
   a. *Strongyloides* b. *Echinococcus granulosus*
   c. *Taenia solium* d. *Trichinella spiralis*
   e. *Echinococcus multilocularis*

10. **Cysticercosis is caused by larva of:** [PGI 99]
    a. *Taenia solium* b. *T. saginata*
    c. *Echinococcus* d. *Ascaris*

11. **Neurocysticercosis, following are true except:**
    a. Not acquired by eating contaminated vegetables
    b. Caused by regurgitation of larva [PGI 98]
    c. Acquired by orofecal route
    d. Acquired by eating pork

12. **Which of the following is the most common location of intracranial neurocysticercosis?** [AIIMS 05]
    a. Brain parenchyma b. Subarachnoid space
    c. Spinal cord d. Orbit

Nematodes

13. **The organism most commonly causing genital filariasis in most parts of Bihar and Eastern UP is:** [AI 03]
    a. *Wuchereria bancrofti* b. Brugia malayi
    c. *Onchocerca volvulus* d. *Dirofilaria*

14. **In which stage of filariasis are microfilaria seen in peripheral blood?** [AI 01]
    a. Tropical eosinophilia
    b. Early adenolymphangiitis stage
    c. Late adenolymphangitis stage
    d. Elephantiasis

15. **A child from Bihar comes with fever. Blood examination shows sheathed microfilaria with nuclei up to tail tip. The diagnosis is:** [AI 00]
    a. *B. malayi* b. *W. bancrofti*
    c. Loa loa d. Onchocerca volvulus

16. **Most common presenting symptom of thread worm infection amongst the following is:** [AI 97]
    a. Abdominal pain b. Rectal prolapse
    c. Urticaria d. Vaginitis

17. **Kalu, 30 year old man, presented with subcutaneous itchy nodules over left iliac crest. On examination they are firm, nontender, and mobile skin scrappings contain microfilaria and adult worms of:** [AIIMS 01]
    a. Loa loa b. *Onchocerca volvulus*
    c. *Brugia malayi* d. *Mansonella persetans*

18. **Larva found in muscle is:** [AIIMS 98]
    a. *Trichinella spiralis* b. *Ancylostoma duodenale*
    c. *Trichuris trichiura* d. *Enterobius vermicularis*

19. **Which of the following parasite does not enter into the body by skin penetration?** [AIIMS 97]
    a. *Dracunculus* b. *Necator americanus*
    c. *Ancylostoma duodenale* d. *Strongyloides*

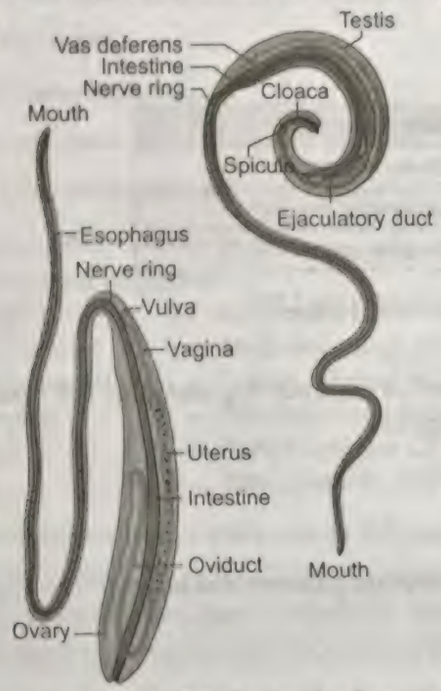
20. **All are true regarding filariasis except:**
    a. Man is an intermediate host [AIIMS 97]
    b. Caused by *Wuchereria bancrofti*
    c. Involves lymphatic system
    d. DEC is used in treatment

21. **Nematodes are differentiated from other worms by:**
    a. Absent fragmentation [PGI 05]
    b. Flat or fleshy leaf-like worms
    c. Separate sexes
    d. Cylindrical body
    e. GIT is formed completely

22a. Parasites penetrating through skin for entry into the body are: [PGI 99; 01]

a. *Ancylostoma duodenale* b. *Strongyloides*
c. Roundworm d. *Trichuris trichiura*

22b. The following are images of an intestinal nematode. Which of these are true about it? [AIIMS 16]



a. Filariform larvae is infective for humans as shown in the diagram
b. Transmitted through contaminated food and water usually
c. Females of these species show parthenogenesis
d. Triclabendazole is the drug of choice.

Miscellaneous

23. All of the following causes biliary obstruction except: [AI 08]

a. *Clonorchis* b. *Ascaris*
c. *Ancylostoma duodenale* d. *Fasciola*

24. Which of the following is not a neuroparasite? [AI 05; PGI 05]

a. *Taenia solium* b. *Acanthamoeba*
c. *Naegleria* d. *Trichinella spiralis*

25. Autoinfection is a mode of transmission in: [AI 00]

a. *Trichinella* b. Cysticercosis
c. *Ancylostoma* d. *Ascaris*

26. Pigs are resevoir for: [AI 00]

a. *T. solium* b. *T. saginata*
c. *Trichinella spiralis* d. Ancyclostoma

27. Sputum examination is not useful in diagnosis of: [AI 98; AIIMS 96]

a. Trichuriasis trichiura b. *Ancylostoma duodenale*
c. *Paragonimus* d. *Strongyloides*

28. Which one of the following does not pass through the lungs? [AI 96]

a. Hookworm b. Ascaris
c. Strongyloidis d. Enterobius vermicularis

29. Which of the following disease is transmitted by egg ingestion? [AI 95]

a. Taeniasis b. Trichinosis
c. Hydatidosis d. Strongyloidosis

30. All the following are true about *Brugia malayi* except:

a. The intermediate hosts in the India are Mansoni
b. The tail tip is free from nuclei [AI 95]
c. Nuclei are blurred, so counting is difficult
d. Adult worm is found in the lymphatic system

31. Autoinfection is seen with: [AIIMS 01]

a. *Ankylostoma* b. *Enterobius*
c. *Echinococcus* d. *Ascariasis*

32. On microscopic examination eggs are seen, but on saturation with salt solution no eggs are seen, The eggs are likely to be of: [AIIMS 99]

a. *Trichuris trichiura* b. *Taenia solium*
c. *Ascaris lumbricoides* d. *Ancylostoma duodenale*

33. Liver is the target organ for: [AIIMS 97]

a. *Fasciola buski* b. *Paragonimus westermani*
c. *Clonorchis sinenses* d. *Schistosoma Hematobium*

34. All float in a saturated salt solution except: [AIIMS 95]

a. *Clonorchis sinensis*
b. Fertilized eggs of ascaris
c. Larva of *Strongyloides*
d. *Trichuris trichiura*

35. Fish acts as intermediate host in: [PGI 04]

a. *D. latum* b. *Clonorchis sinensis*
c. *H. diminuta* d. *H. nana*

36. Parasites causing lung infestation are: [PGI 03]

a. *H. nana* b. *Paragonimus westermani*
c. *Taenia saginata* d. *E. granulosus*
e. *E. multiocularis*

37. Larval form in stool is found in: [PGI 01]

a. *Strongyloides* b. *Ancylostoma duodenale*
c. *Ascaris lumbricoides* d. *Necator americanus*
e. *Trichuria*

38. Autoinfection is seen with: [AIIMS May 09]

a. *Cryptosporodium* b. *Strongyloides*
c. *Giardia* d. *Gnathostoma*

39. Visceral larva migrans caused by: [AI 2011]

a. Strongyloides b. Toxocara canis
c. Ankylostoma d. Dirofilaria

40. Small Intestine helminth are: [PGI-11]

a. Ascaris b. Necator
c. Trichirus d. Enterobius
e. Ancyclostoma

41. Hydrocele and edema in foot occur in:

a. W. Bancrofti b. B. Malayi
c. B. Timori d. Oncocerca volvulus
e. Guinea worm [PGI Nov. 2011]

42. Filarial stage of adult worms responsible for diseases in all of the following except: [AIIMS May 2015]

a. Onchocerca volvulus b. Brugia
c. Wuchereria d. Mansonella ozzardi

Explanations and References with Illustrative Answers

1. **Ans. (d) None** *Ref. Paniker 7/e, 149*

Name of Parasite	Associated cancer
• Clonorchis sinensis	– Cholangiocarcinoma
• Opisthorchis viverrini	– Cholangiocarcinoma
• Schistosoma hematobium	– Bladder cancer (SCC)

2. **Ans. (a) *Diphyllobothrium latum*** *Ref. Paniker 7/e, p 115; Harrison 19/e, p 1434, 18/e, 1765*

Diphyllobothrium (Fish Tapeworm = Broad Tapeworm)

- ***Definitive host*** – Adult worm lives in small intestine *(usually in the ileum)* of man, cat, dog and other fish eating mammals.
  Infective stage for man – Third stage larva/Plerocercoid larva/sparganum.
- ***Intermediate host***
  First – Cyclops infected by coracidium larva.
  Second – Freshwater fish infected by procercoid larvae.
- *Eggs* – Operculated, shed in feces but are not infective to man.
- ***Mode of infection*** – Ingestion of imperfectly cooked infected fish containing plerocercoid larva.
- **Clinical features:**
  - Mechanical obstruction
  - Abdominal discomfort
  - Diarrhoea
  - Nausea
  - Anaemia (pernicious type called as ***Bothriocephalus Anaemia***) which is due to vitamin B_{12} deficiency.
- **Diagnosis:** Demonstration of eggs in feces.
- **Treatment:** Praziquantel/Niclosamide.

3. **Ans. (b) *Clonorchis* infestation** *Ref. Paniker 7/e, p 148*

Clonorchis Sinensis

- Also called as ***Chinese liver fluke***
- Human clonorchiasis occurs in Japan, Korea, Taiwan etc.
- ***Host*** – *Definitive:* Human
  - *Reservoir:* Dogs and other fish eating canines
  - *Intermediate:* First is snail and second is fresh water fish.
- ***Life cycle*** – Adult worm lay eggs in biliary tract (brown shell, operculum at one pole and small hook like spine at other pole) which passed in feces
  ↓
  Eggs containing ciliated miracida are ingested by snail
  ↓
  Miracida converts into cercaria and escape from snail
  ↓
  Cercaria attached to 2nd intermediate host and encyst to become metacercariae (infective stage for humans)
- ***Mode of infection:*** – Ingestion of raw or inadequately processed fish or contaminated fingers/utensils used for cooking for fish.
  - Metacercariae excyst in duodenum of human and enter CBD through ampulla of Vater.
- ***Pathogenicity:*** – Cystic dilatation/obstruction of CBD causing cholangitis/Jaundice/calculus formation/fever/epigastric pain/diarrhea/tender hepatomegaly
  - Biliary cirrhosis and portal hypertension
  - Cholangiocarcinoma/increased tendency to become biliary carriers of typhoid bacilli.
- ***Diagnosis:*** – Demonstration of eggs in feces or aspirated bile
  - Liver imaging can show eggs/dilated biliary tract.

Remember: Infection of ***fasciolopsis buski/giant intestinal fluke (largest trematode)*** present with partial obstruction of bowel, diarrhea, abdominal pain, oedema, ascitis, anemia, prostration and persistent diarrhea.

Gnathostoma spinigerum/G.hispidium cause indurated nodules or abscess or creeping eruption (larva migrans) or wandering larvae may damage brain/eyes. Humans are infected by ingestion of fish containing third stage of larvae but worm does not develop further. So humans are *paratenic host.*

4. **Ans. (a)** ***Clonorchis sinensis*** *Ref. Paniker 7/e, p 149*

Already explained

5. **Ans. (a)** ***Clonorchis sinensis*** *Ref. Pankier 7/e, p 149*

Already explained

6. **Ans. (a)** ***Clonorchis sinensis*** *Ref. Paniker 7/e, p 149, 225*

Angiostrongylus cantonensis

Also called as rat lungworm

Natural host	– Rat, crabs, fresh water prawns and frogs
Intermediate host	– Molluscs, slugs, and snails
Mode of transmission	– Eating infected molluscs etc. containing third stage larva
Pathogenicity	– Meningoencephalitis by died larvae, so antihelminthic is not recommended
Diagnosis	– Peripheral and CSF eosinophilia
	– Demonstration of larvae/adult worm in CSF

Angiostrongylos costaricensis causes inflammation of lower bowel as abdominal angiostrongyliasis

Remember: H. diminuta (rat tapeworm) is common parasite of rat and mice. Similar to H. nana in its life cycle but is larger (10-60 cm) than H. nana. Human infection is rare, asymptomatic, occur by accidental ingestion of infected rat fleas.

A. Cantonensis is the commnest cause of cosinopholic miningitis.

7. **Ans. (b)** ***Taenia solium*** *Ref. Paniker 6/e, p 144 - 145, 7/e, p 120 - 121*

Taenia Solium (Pork Tape Worm)

- Causative agent of ***cysticercosis.***
- ***Definitive host*** = Adult worm lives in jejunum of man.
  Infective stage for man:
  - Pork's flesh containing cysticercus cellulosae larvae or bladder worm.
  - Also by eggs either by ingestion in water/vegetables; Autoinfection by fingers contaminated with eggs from perianal skin or feces and retrograde peristalsis.
- ***Intermediate host*** = Pig
  Infected stage for pig = Eggs containing hexacanth larvae, so eggs are infective for both man and pig.

Remember:
- Larvae is found in both man and pig.
- But in man it is dead end Infection.
- Infection occur in both vegetarian and non vegetarian.

Taenia saginata

- Causative agent of *Cysticercosis bovis.*
- ***Definitive host*** = Adult worm lives in jejunum of man.
  Infected stage for man – Undercooked beef containing *Cysticercus bovis* larvae in striated muscle.
- ***Intermediate host*** = Cow/bufflao = harbors larval stage.
  Infective stage of intermediate host = Eggs containing oncosphere during grazing.

Remember:
- Larva is absent in man.
- Eggs does not infect man.
- Infection does not occur in vegetarians.
- MC site involved in man is striated muscles particularly muscles of tongue, neck, shoulder, and myocardium.

Trichuris trichiura* = *Whipworm

- Life cycle in ***one host only*** (monoxenous).
- Adult worm lives in cecum and appendix ***(Large intestine)***.
- ***Mode of infection:*** Feco-oral when mature embryonated eggs containing infective rhabditiform larva are swallowed in food or water.

Remember: All nematodes are monoxenous (one host) except *T. spiralis*, Filaria, Guinea worm.

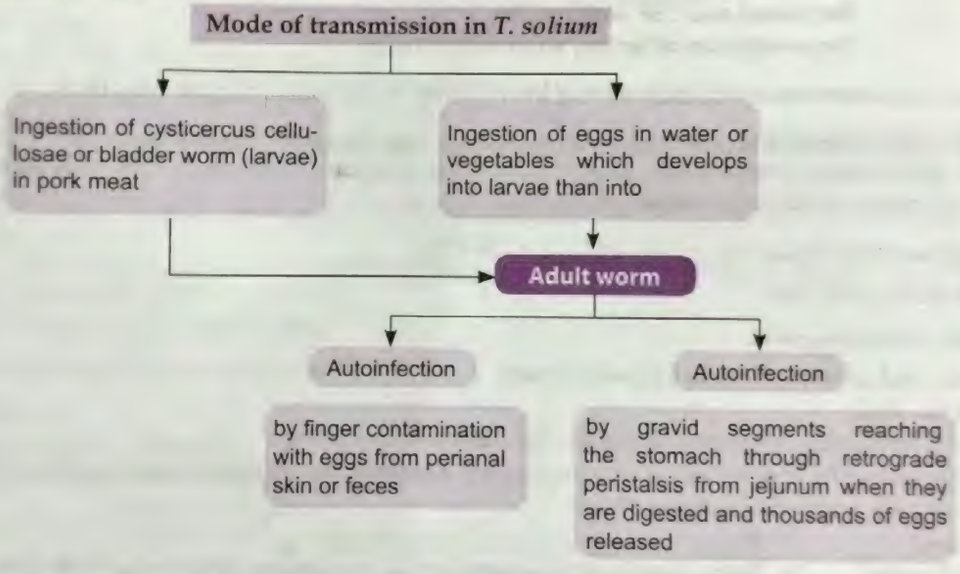
***Echinococcus granulosus* = Dog or Hydatid tape worm**

- Causative agent of **cystic or unilocular hydatid disease.**
- ***Definitive host*** – Dog and other canine carnivora.
  Infective stage – Fertile hydatid containing fully developed scolex.
- ***Intermediate host*** – Sheep and man (***Dead end*** in man).
  Infective stage – Egg during grazing or ingestion of Eggs passed by infected dogs.

Remember: Alveolar or multilocular hydatid disease is caused by *E.multilocularis*.

8. **Ans. (b) Cysticercosis** *Ref. Harrison 18/e, p 1761; 19/e, p 902*
"Neurocysticercosis is the most common parasitic disease of CNS worldwide."

Neurocysticercosis = Cysticercosis of CNS



- So man is ***both*** definitive and intermediate host for *T. solium* but in man it is Dead end infection.
- Adult tape worm lives in the human intestine usually in the jejunum

Clinical features:

- *MC* manifestation is new-onset partial seizures with or without secondary generalization.

Site		Symptoms	
•	Brain parenchyma ***(MC)***	:	Seizures or focal neurological defects
•	Subarachnoid or ventricular space	:	Increased intra-cranial tension
•	Spinal cyst	:	Mimic intraspinal tumour

Diagnosis: – By non-contrast CT.
– *MC* finding on neuroimaging is parenchymal brain calcification.

Treatment: Antiepileptic therapy + Albendazole or praziquantel.

Remember: Site of cysticercosis – CNS > Subcutaneous tissue > globe of eye.

9. **Ans. (b) and (e) *Echinococcus granulosus* and *Echinococcus multilocularis*** *Ref. Paniker 7/e, p 127*
Already explained

10. **Ans. (a) *Taenia solium*** *Ref. Harrison 19/e, p 902; 18/e, p 1760*
Already explained

11. **Ans. (a) Not acquired by eating contaminated vegetables** *Ref. Paniker 7/e, p 128*
Already explained

12. **Ans. (a) Brain parenchyma** *Ref. Harrison 19/e, p 902; 18/e, p 1761*
Already explained

13. **Ans. (a) *Wuchereria bancrofti*** *Ref. Paniker 7/e, p 205 - 206; Harrison 19/e, p 1417; 18/e, p 1745*

Filariasis

- *Definitive host* – man.
- *Intermediate host* – insects
- ***Genital filariasis*** is type or manifestation of lymphatic filariasis.
- Lymphatic filariasis is caused by Brugia and *Wuchereria bancrofti* so ***"c"*** and ***"d" choices are ruled out.***
- Lymphadenitis and Lymphangiitis involve both upper and lower extremities in both bancroftian and *brugian filariasis* but involvement of genital lymphatics occur almost exclusively with *W. bancrofti* and brugian rarely involved genitalia.

	Parasite	Location in body		Characteristics of Microfilaria	Principal vector	Clinical Features	DOC
		Adult	Microfilaria				
I. Lymphatic Filariasis							
	Wuchereria bancrofti	Lymphatic	Blood	Nuclear column discrete, Sheathed, pointed tail tip free of nuclei	*Culex quinque fasciatus* (culex fatigans)	Asymptomatic or subclinical microflaremia; hydrocele; acute adenolymphangitis (high fever, lymphatic inflammation, local edema.	Dec
	Burgia malayi	Lymphatic	Blood	Sheathed, blunt tail tip with two terminal nuclei, nuclear column blurred	*Mansonia spp*	Chronic lymphatic disease (elephantiasis); funiculitis; scrotal	
	Burgia timori	Lymphatic	Blood	Sheathed, longer than *Mf malayi*	*Anopheles barbirostris* (Not in India) Mansonia	Pain and tenderness	
II. Subcutaneous filariasis							
	Loa loa	Connective tissue, conjunctiva	Blood	Sheathed, nuclei extending up to pointed tail	*Chyrsops spp*	Recurrent transients subcutaneous swelling is fugitive or calabar swellings, ocular manifestation	Dec
	Onchocerca volvulus= Convulated = binding filaria	Subcutaneous nodules	Skin, eyes	Unsheathed, blunt tail tip free of nuclei	*Simulium spp.*	Pruritus, and rash (MC; Iverm palpable subcutaneous ectin nodules; lymphadenopathy; visual impairment (River blindness)	Iverm-ectin
	Mansonella streptocerca	Subcutaneous	Skin	Unsheathed; blunt tail tip with nuclei	Culicoides	Pruritus, papular rash, Iverm-pigmentation; inguinal ectin, adenopathy	Dec Iverm-ectin
III. Serous cavity filariasis							
	Mansonella ozzardi	Peritoneum and pleura	Blood	Unsheathed pointed tail tip without nuclei	Culicoides	Headache articular Iverm pain, fever, pulmonary ectin symptoms, adenopathy, hepatomegaly, pruritus, eosinophilia	Iverm-ectin
	Mansonella perstans	Peritoneum; and pleura, mesentery, peri-renal tissue	Blood	Unsheathed, pointed tail tip with nuclei	Culicoides	Asymptomatic mostly; transient angioedema; pruritus of arm, face; fever; headache; arthralgia; right upper quadrant pain	Dec

DEC: Diethylcarbomazine

14. Ans. (b) Early adenolymphangiitis stage *Ref. Park 21/e, p 246; Paniker 7/e, p 205; Chatterjee 12/e, p 195 - 197*

Clinical manifestations			
Lymphatic filariasis			
i.	*Asymptomatic amicrofilaremia*	–	No microfilarae and no clinical manifestation.
ii.	*Asymptomatic microfilaremia*	–	Positive for Mf without any symptoms.
iii.	*Stage of acute manifestation*	–	Recurrent episodes of acute inflammation in lymph glands and vessels characterized by lymphadenitis, lymphangitis, filarial fever, lymphangiovarix and lymphorrhagia. It is caused by adult worm but blood ***may reveal microfilarae in early phase.***
iv.	*Stage of chronic obstructive lesions*	–	Caused by adult worm blocking lymph nodes and vessels either mechanically or are commonly due to allergic inflammatory reactions to worm antigens and secretions. Granuloma may form.
		–	Main features are hydrocele, elephantiasis (***MC*** site is leg), Lymphedema ***(non pitting or brawny edema).***
		–	Microfilaria in blood are absent either due to death or their failure to reach the systemic circulation due to lymphatic obstruction.
Occult filariasis			
	Meyers Kouwenaar syndrome	–	It is due to hypersensitivity reactions to filarial antigens. It ***includes Tropical pulmonary eosinophilia*** characterized by nocturnal paroxysmal cough, wheeze and blood eosinophil count is above 3000 per cmm.
		–	***Microfilariae*** are ***not usually*** detectable in blood but lung biopsies may show microfilariae
		–	Antifilarial antibody titres are characteristically elevated

15. Ans. (a) *B. malayi* *Ref. Paniker 7/e, p 214; Park 21/e, 244*

- Heavily infected areas of lymphatic filariasis are found in Uttar Pradesh, Bihar, Jharkand, Andhra Pradesh, Orissa, Tamil Nadu, Kerala and Gujarat. So, it is case of lymphatic filariasis.
- ***Sheathed microfilaria are:***
  - Loa-loa
  - *Burgia malayi*
  - *W. bancrofti*

  ] Tail tip has nuclei but loa-loa cause subcutaneous filariasis found in Africa (not in Bihar.) - Tail tip free of nuclei.

Mnemonic: Low (L); Birth (B); Weight (W)

Features	Mf bancrofti	Mf. malayi
Length	250-300 µm (longer)	175-300 µm
Appearance	Graceful sweeping curves	Kinky, with secondary curves
Cephalic space	Length and breadth equal	Almost twice as long as broad
Stylet at anterior end	Single	Double
Excretory pore	Not prominent	Prominent
Nuclear column	Discrete nuclei	Blurred/smudged
Tail tip	Pointed, free of nuclei	Kinkled and 2 distinct nuclei one at tip, the other subterminal
Sheath	Faintly stained	Well stained

16. Ans. (a) Abdominal pain *Ref. Paniker 7/e, p 190 - 192; Harrison 19/e, p 1416; 18/e, p 1743*

Enterobius vermicularis = Pinworm = thread worm = seat worm

- Life cycle in ***one host only (man).***
- *No* intermediate host.
- Adult worm live in caecum, appendix and adjacent part of ascending colon (i.e. in large intestine).
- *Mode of infection* – By ingestion of egg containing infective larvae (=autoinfection) and retroinfection.
- **Clinical features:**
  - Mostly asymptomatic.
  - *Cardinal symptoms:* Perianal pruritus (It is nocturnal and cause excoriation + bacterial superinfection).
  - Abdominal pain and weight loss.
  - Vulvovaginitis and pelvic or peritoneal granuloma.

- **Diagnosis:**
  - Fecal examination is not useful.
  - Apply clear cellulose acetate tape to perianal region in the morning and see characteristic ***planoconvex, non- bile stained*** pin worm eggs (containing tadpole shaped coiled embryo) on microscopy.
  - Sampling can also done by NIH swab, scotch tape, glass pestle swab.
- **Treatment:**
  - Mebendazole/Albendazole/Pyrantel pamoate.

17. **Ans. (b) Onchocerca volvulus** *Ref. Paniker 7/e, p 216 - 217*

- *It is a case of subcutaneous filariasis* so causes of lymphatic filariasis (Brugia) and serous cavity filariasis (M.perstans) are ruled out. Thus we left with only 2 options:

Loa Loa = Eye Worm: Cause Loiasis/Fugitive Swellings Or Calabar Swellings
- ***Definitive host*** = Man (in subcutaneous tissue)
- ***Intermediate host*** = Vector – day biting flies (Chyrysops)
- ***Mode of transmission:*** Bite of infected Chyrysops
- ***Microfilariae:*** Are sheathed and show diurnal periodicity and appear in blood only during day and taken by chyrysops in which Mf develop into infective third stage larvae.
- Clinical features: Is due to migration of adult worms causing fugitive swellings (which disappear in few days only to reappear elsewhere); Ocular manifestations.
- **Diagnosis** : Demonstration of adult worm from scraping of skin or conjunctiva.
- **Treatment** : Surgery to remove worms; DEC; corticosteroids

Onchocerca Volvulus:
- Convulated or blinding filaria causing ***onchocerciasis or river blindness.***
- It is 2nd major cause of blindness in the world.
- **Vector:** Day biting female black flies (are pool feeders) Simulium, which breed in fast flowing rivers.
- ***Microfilariae:*** Unsheathed; non-periodic; found in skin, subcutaneous lymphatics, conjunctiva and rarely in blood.
- **Clinical features:** Subcutaneous nodule or onchocercoma which is circumscribed, firm, non-tender tumor tend to occur over anatomical sites where bones are superficial such as scalp, scapula, ribs, elbow, iliac crest, sacrum, knees.
- Lesions in skin (pruritus, pigmentation, atrophy, fibrosis) and eyes (photophobia to blindness, glaucoma, punctate or sclerosing keratitis, iridocyclitis, glaucoma) also seen.
- **Diagnosis:** Demonstrating microfilariae by slicing off a silver of skin; aspirating subcutaneous nodules, conjunctival biopsies.
- **Treatment:** *Ivermectin; Enucleation of nodules; DEC (cause Mazotti reaction) and Suramin.*

18. **Ans. (a) *Trichinella spiralis*** *Ref. Paniker 7/e, p 168*

Classification of Nematodes on the basis of Mode of infection.

Ingestion	Penetration of skin	By blood sucking insect	Inhalation of dust containing eggs
• *Eggs* – *Ascaris* – *Enterobius* – *Trichuris*	• *Ancylostoma* • *Necator* • *Strongyloides*	• Filariae	• *Ascaris* • *Enterobius*
• *Larvae within Intermediate host or drinking water containing cyclops* – Dracunculus	**Mnemonic: ANS**		
• *Encysted larvae in muscle:* Trichinella			

19. **Ans. (a) Dracunculus** *Ref. Paniker 6/e, p 159*

In dracunculus, human get infected by drinking unfiltered water containing infected cyclops

20. **Ans. (a) Man is an intermediate host**

Already explained

21. Ans. (a, c, d) and (e) Absent fragmentation, Separate sexes Cylindrical body and GIT is formed completely
Ref. Paniker 6/e, p 113 - 114

Features	Nematodes	Trematodes	Cestodes
Shape	Cylindrical/thread	Flat or fleshy leaf-like or flukes	Tape like
Segmentation	Unsegmented	Unsegmented	Segmented
GIT	Complete	Incomplete	Absent
Suckers	Absent	Present	Present
Hooks	Absent	Absent	May present
Sex	Separate (Dioecious)	Monoecious except Schistosomes	Monoecious
Number of host	Monoxenous except *Trichinella*, filarial, *Dracunculus medinensis*	Digenetic	Digenetic except *H.nana*
Body cavity	Present	Absent	Absent

Remember: Nematodes may be viviparous or oviparous or Ovo-viviparous but other worms are oviparous

22a. Ans. (a) and (b) *Ankylostoma duodenale* and *Strongyloides* *Ref. Paniker 6/e, p 159*

Already explained

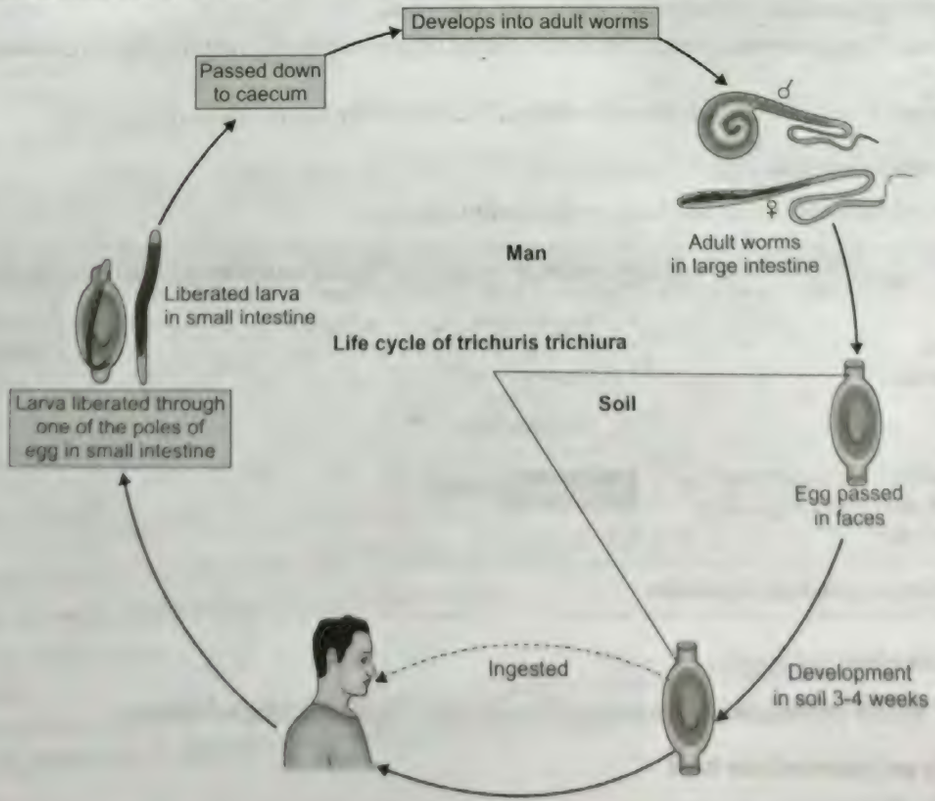
22b. Ans. is (b) i.e. *Transmitted* *Ref. Paniker 7/e, p 173*

This is the figure denoting longitudinal section of trichuris tricuria as characterized by coiled posterior end of male.
Now let us consider each option

Option "a"
Infective form is rhabditiform larvae.

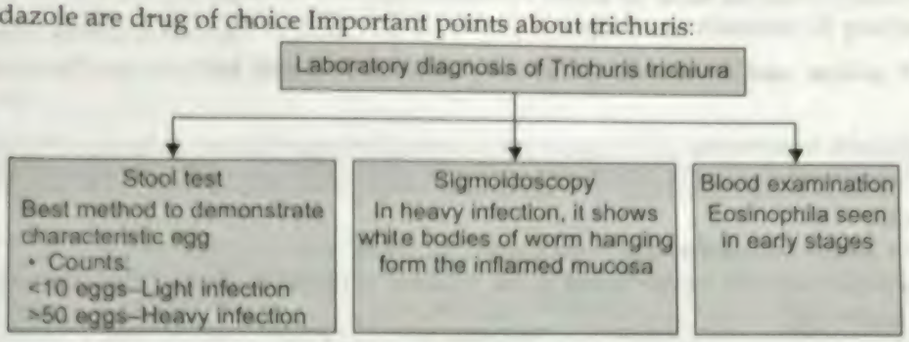
Option "b"
Mode of transmission: Infection occurs in human when mature embryonated eggs containing the infective larvae are swallowed in contaminated food or water.

Option "c"
Pathogenicity: Usually asymptomatic, however when worm load is heavy, disease may result either due to mechanical effects or allergic reactions.
The whip like anterior portion of worm (both male & female) usually attaches it self to the mucosa of caecum and upper colon.
One worm is believed to suck about 0.005 mL of blood.



Option "d"

Mebendazole or albendazole are drug of choice Important points about trichuris:



23. Ans. (c) Ankylostoma *Ref. Harrison 17/e p 1334, 1335, 2000*

Hepatobiliary Parasitism

- Common in Southern China
- Biliary tract is infestated by adult helminths or their ova
- ***Causes:***
  (a) Trematodes (Liver or biliary flukes) *MC*
  - *Clonorchis sinensis*
  - *Opisthorchis viverrini* or *O. felineus*
  - *Fasciola hepatica*

  (b) Nematode (*Ascaris lumbricoides* by intraductal migration of adult worm)
  (c) Cestodes (*Echinococcus* by intrabiliary rupture of hydatid cyst)
- ***Clinical Features:***
  - Chronic/recurrent pyogenic cholangitis ± multiple hepatic abscess
  - Ductal stones
  - Biliary obstruction
- ***Diagnosis:***
  - Cholangiography
  - Characteristic ova on stool examination
- ***Treatment:*** If obstruction is present, TOC is laparotomy under antibiotic coverage with common duct exploration and biliary drainage procedure.

Helminths causing obstructive jaundice are: *Ascaris, Clonorchis, Fasciola, Echinococcus.*

24. Ans. (d) *Trichinella spiralis*

Brain Parasites							
Protozoa		Helminths					
		Larvae of cestodes		Nematodes		Ecotopic ova	
–	*E. histolytica*	–	Cysticercus cellulosae (*T. solium*)	–	Visceral larva migrans	–	*F. hepatica*
–	*Naegleria*	–	Hydatid cyst (*E. granulosus*)	–	*Ascaris lumbricoides*	–	*Heterophyes hyterophyes*
–	*Acanthamoeba*	–	*Multiceps* sp	–	*Strongyloides stercoralis*		*Schistosoma hematobium*
–	*Trypanosoma*	–	*Toxoplasma gondii*	–	*Gnathostoma spinigerum*		
–	*P. falciparum*						

25. Ans. (b) Cysticercosis *Ref. Paniker 7/e, p 4*

Autoinfection is seen in:

C = *Capillaria philippinensis*
H = *Hymenolepis nana*
E = *Enterobius vermicularis*
S = *Strongyloides stercoralis*
T = *Taenia solium* (cysticercosis)
C = Cryptosporidium Parvum

Mnemonic: CHEST congestion

- Autoinfection in cysticercosis occur by finger contamination with the eggs from the perianal skin or feces and by gravid segments reaching the stomach by retrograde peristalsis from jejunum.

26. Ans. (a) and (c) *T. solium;* and *Trichinella spiralis* *Ref. Paniker 7/e, p 123, 167*

Taenia solium

- Also known as ***pork taperworm.***
- Intermediate host is pig.

Trichinella spiralis

- ***Primary host*** = Man – Mucosal epithelium of small intestine.
- ***Infective form*** is the encysted larvae found in the muscles of pig and other animals (larvae is tissue parasite).
- ***Intermediate host*** = Pig
- ***Mode of Infection*** – Uncooked pork meat containing encysted larvae.
- Female worm is viviparous (eggs are absent).
- Human infection is dead end infection.

27. Ans. (a) *Trichuris trichiura* *Ref. Paniker 7/e, p 172, 238*

- Sputum examination can be useful for any parasite which pass through lungs at some or other stage during their life cycle.
- **These parasites are:**
  - Rhabditiform larva of Ascaris
  - Golden brown eggs of *Paragonimus westermani* (Lung fluke)
  - Migrating larvae of *Strongyloides, Ancylostoma, Ancylostoma ducdenale, nector americanus*
  - *Entamoeba histolytica* - Chocolate brown sputum due to hepatobronchial fistula.

28. Ans. (d) *Enterobius vermicularis* *Ref. Paniker 7/e, p 190, 157*

Already explained

29. Ans. (c) Hydatidosis *Ref. Paniker 6/e, p 159*

Following parasitic infection occur by ingestion of eggs:

- *Echinococcus granulosus*
- *Hymenolepis nana*
- *Ascaris*
- *Trichuris*
- *Enterobius*
- *Taenia solium (but mainly transmit by ingestion of undercooked pork meat containing cysticercus cellulose).*

Mnemonic: Esha **HATE** Tushar

30. Ans. (b) The tail tip is free from nuclei *Ref. Paniker 6/e, p 196*

Microfilaria tail free of nuclei

- *W. Bancrofti*
- *O. Volvulus*
- *M. Ozzardi*

Mnemonic: BOO

31. Ans. (b) *Enterobius* *Ref. Paniker 7/e, p 190*

Already explained

32. Ans. (b) *Taenia solium* *Ref. Paniker 7/e, p 173*

Helminths whose eggs float in saturated salt solution	Helminths whose eggs do not float in the saturated solution
• Enterobius vermicularis	• Eggs to Taenia solium and Taenia saginata
• Ancylostoma duodenale	• Eggs of all intestinal flukes
• Necator americanus	• Unfertilized eggs of Ascaris lumbricoides
• Ascaris lumbricoides	
• Trichuris trichiura	

33. Ans. (c) *Clonorchis sinensis* *Ref. Paniker 7/e, p 148*

- All are trematodes *(also known as flukes)*.
- Man is definitive and snails are intermediate host usually.
- They are classified as:

FLUKES		HABITAT	INTERMEDIATE *HOST*	MODE OF TRANSMISSION
a. Blood Flukes				
i.	*Schistosoma (Bilharzia) hematobium*	Vesical and pelvic vein plexuses	Snail which is infected by miracidium	– Water borne disease – Man is infected by bathing in contaminated water when cercaria penetrates unbroken skin
ii.	*S.mansoni*	Inferior mesentric vein (Intestinal bilharziasis) or Schistosomal dysentry		
iii.	*S.japonicum or oriental schistosomiasis*	Superior mesenteric vein (katayama disease)		
b. Liver Flukes				
i.	*Clonorchis sinensis (=Chinese liver flukes)*	Biliary tract (associated with Cholangiocarcinoma)	1st intermediate host: snail 2nd intermediate host: fish	Fish containing metacercariae are eaten raw or inadequately processed
ii.	*Fasciola hepatica (=sheep liver fluke)*	Biliary tract	Primary host: man/sheep Intermediate host: snails	Ingestion of watercress or other water vegetation containing metacercaria
c. Intestinal Flukes				
i.	*Fasciolopsis buski (Giant intestinal fluke)*	Duodenum or jejunum	Molluscum Snails	Ingestion of roots of lotus, bulb of water chesnut and other acquatic vegetations
ii.	*Heterophyes*	Small intestine	Molluscum Snails	Ingestion of fishes since cercriae encyst on fishes
iii.	*Metagonimus Yokogawai*	Small intestine	1st intermediate host: fresh water snail; 2nd intermediate host: fish	Ingestion of raw fish
v.	*Gastrodiscoides hominis (only fluke inhabiting human large intestine)*	Large intestine	Molluscum	Ingestion of water plants
d. Lung Fluke				
	Paragonimus Westermani = Oriental lung fluke	Cystic space of lung	1st intermediate host: snail 2nd intermediate host: fresh water cray fish or crab fish	Inadequately cooked crabs, cray fish

34. Ans. (a) *Clonorchis sinensis*

Important Points about clonorchis sinensis

- Flat transparent hermaphroditic worm.
- Human are the principal definitive host
- Snail and fish are respective **intermediate** host
- Adult worm may cause obstruction and blockage of the common bile duct leading to cholangitis
- Few cases go to biliary cirrhosis and portal hypertension
- Associated with cholangiocarcinoma
- Human acquired infection by **eating undercooked fresh** water fish carrying metacercariae larva (**infective stage**)
- Treatment: Praziquantel.

35. Ans. (a) and (b) *D. latum* and *Clonorchis sinensis* *Ref. See below*

Two intermediate host with fish as one of them are seen in:

Parasite	Ist intermediate host	IInd intermediate host
i. *Paragonimus westermani* (Trematode)	Snail	Fresh water cray fish or crab
ii. *Clonorchis sinensis* (Trematode)	Snail	Fish
iii. *Metagonimus yokogawai* (Trematode)	Fresh water snail	Fish
iv. *Diphyllobothrium latum* (Cestode)	Cyclops	Fresh water fish

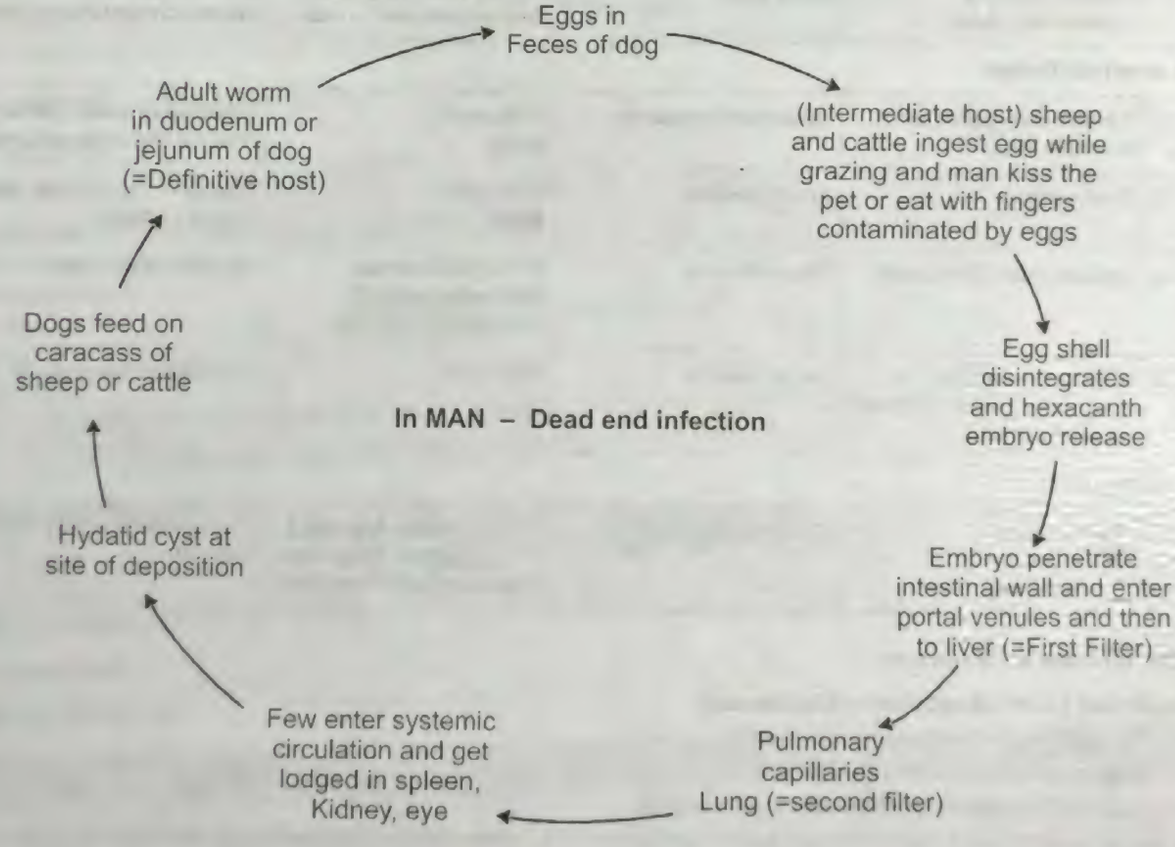
Remember:
- All cestodes are digenetic (require 2 host) except *H. nana*.
- Intermediate host for *H. diminuta* is Rat flea.

36. Ans. (b, d and e) *Paragonimus westermani, E. granulosus* and *E. multiocularis* *Ref. Paniker 6/e, 150 - 1555*

- *Taenia saginata* and *H. nana* are intestinal cestodes.
- Paragonimus is lung fluke, so there is no doubt about this.

Clinical manifestations of hydatid cyst

- *MC* site of Hydatid cyst (*E. granulosus* typically develops unilocular cyst): ***Liver***; mostly in right lobe presenting as Hepatomegaly, pain, obstructive jaundice.
- ***Next common*** site is ***lung*** usually in the lower lobe of right lung – cough, hemoptysis, chest pain, dyspnea.
- Hypersensitivity, fatal anaphylaxis if cyst ruptures.
- Kidney - pain, hematuria.
- Osseus hydatid.



Life cycle of E. Granulosus

E. Multilocularies:

- *Cause alveolar or multilocular hydatid disease in man.*
- ***Definitive host:*** Foxes, dogs, cats

- *Intermediate host*: Rodents (main), man
- Man infected by eating fruits or vegetables contaminated with feces.
- *Clinical features:*
  - *MC* site liver: Multilocular infiltrating lesion mistaken for malignant tumor.
  - Also metastasize to lungs and brain.
- *Treatment*: Resection is *TOC*.

Remember: Malignant hydatid Disease:
- Caused by Echinococcus multilocularis. Present with multiple small cysts in both lobe of liver
- Mimics malignancy and most patient die of liver failure

37. Ans. (a, b) and (d) Strongyloides, *Ancylostoma duodenale* and *Necator americanus* *Ref. See below*

Nematodes		Fecal examination
i.	*Trichinella spiralis*	Adult worm
ii.	*Trichuris trichiura*	Eggs (barrel shaped)
iii.	*Strongyloides*	Rhabditiform larvae
iv.	*Ancylostoma duodenale and necator americanus*	Egg which may hatched, so rhabditiform larvae can also seen
v.	*Enterobius vermicularis*	Usually not useful
vi.	*Ascaris lumbricoides*	Eggs and adult worm (*Larvae in sputum or gastric washings)
vii.	*Filariasis*	No role
viii.	*Dracunculus medinensis*	No role (* tip of worm projects from base of ulcer)

38. Ans (b) *Strongyloides* *Ref. Paniker 7/e, p 4*

Already explained

39. Ans (b) Toxocara canis *Ref. Paniker 7/e, p 162 - 166*

Larva migrans occur when larvae lose their normal pathway and wander around aimlessly

It generally seen when human infection occur with non human species of nematodes so larvae is unable to complete its normal development . It can also occur when parasitic nematodes infect immune person, so that its immunity does not allow normal progression of infection.

It can be of two types:

Larva Migrans

Cutaneous larva migrans
Larva migrans takes place in skin)

- Also called as creeping eruption
- Caused by nematode larvae that infect by skin penetration

Causes include:
- Ancylostoma brazilense (MC)
- *A. caninum*
- Necates americanus
- Loa loca
- Ganasthoma spinigerum

Visceral larva migrans
(Larva migrans occurs in deeper tissues)

- Caused by migration of larvae of nonhuman species of nematodes that infect by oral route

Causes include:
- Toxocara canis (MC)
- Toxocara cali
- Anisakis
- A. lumbricoides
- Ganasthoma spinigerum

Clinical features

- Gnathostomiasis produce lesion in deeper subcutaneous tissue or in muscles
- Strongyloides stercoralis causes larava currens (= rapidly moving lesion)
- Loa loa, Dirofilaria Fasciola, paragonimus causes creeping lesion of abdominal wall
- Hypoderma and Gastrophilus causes creeping myiasis

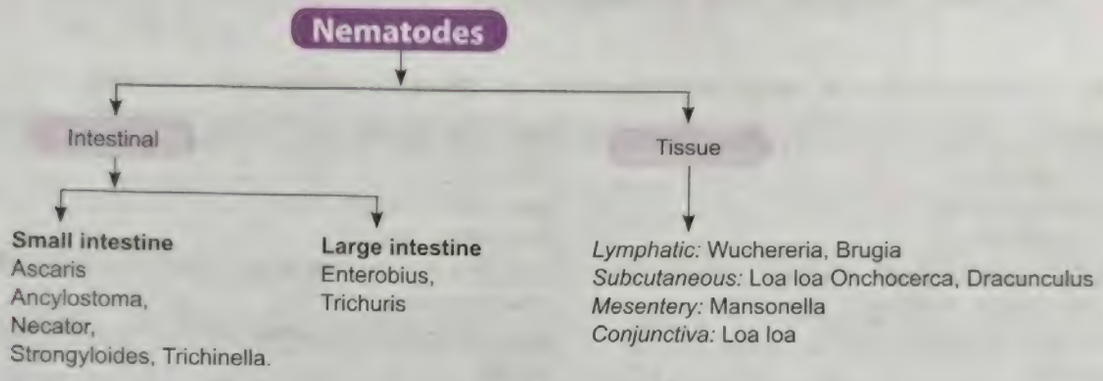
Fever, hepatomegaly, pneumonitis, hyperglobal anemia, pica, neurological disturbances (called as neural larva migrans) and endophthalmitis (Ophthalmic larva migrans)

Diagnosis by passive hemoglutination, bentonite flocculation, micropreapitation, ELISA

Treatment is thiabendazole or deworming of household pets

Remember: Rhabditiform larva: First stage feeding larvae found in Strongyloides stercolis, Ancylostoma duodenale, Necater americanus.
It is non-infective
Filiform larvae : Non feeding infective larvae

40. **Ans (a, b, e) Ascaris, Nicator, Ancyclostoma** *Ref. Paniker 6/e, p 159*



41. **Ans. (a) W. Boncrofti** *Ref.: Paniker 7/e p 209*

Hydrocele is seen only in Bancroftian filariasis, not in Brugian filariasis.

42. **Ans. (d) Mansonella ozzardi** *Ref.: Paniker 7/e p 219*

Mansonella Ozzardi

M. ozzardi is a new world filaria seen only in central and south America and the West Indies.

- The adult worms live in the **body cavities** of humans, mainly in **peritoneum**, less often in **pleura**, and rarely in **pericardium**.
- The microfilariae are unsheathed and nonperiodic.
- Vectors are *Culicoides* species.
- infection does not cause any illness.

Chapter Review

1. **Which is false about *Wuchereria bancrofti*?** [AI 90]
   a. Causes filariasis
   b. Body is long and slender
   c. Terminal nuclei absent
   d. Man and anopheles are host
   [Ref. Paniker 7/e, p 205, 206]

2. **Schistosoma Japonicum resides in:** [AI 92]
   a. Vesical plexus b. Splenic vein
   c. Systemic circulation d. Gall bladder
   [Ref. Paniker 7/e, p 146]
   S. japanicum lives in superior mesenteric vein and its branches.

3. **Definitive host for Guinea worm is:** [AIIMS 93]
   a. Man b. Cyclops
   c. Snail d. Cyclops and man
   [Ref. Paniker 7/e, p 221]

4. **The following infection resembles malignancy:** [JIPMER 02]
   a. *Echinococcus granulosus*
   b. *E. multilocularis*
   c. *E. vogeli*
   d. *E. oligarthus* *[Ref. Paniker 6/e, p 155]*

5. **Which of the following parasites is infectious as it is passes from stool?** [UP 02]
   a. *Ancylostoma duodenale*
   b. *Enterobius vermicularis*
   c. *T. solium*
   d. *Ascaris* *[Ref. Paniker 7/e, p 192]*

6. **All are true about *Brugiamalayi*, except:** [Delhi 02]
   a. Nucleated tail tip
   b. Enveloped sheath
   c. Nocturnal periodicity
   d. Smooth curved in stain preparation
   [Ref. Paniker 6/e, p 196, 208]

7. **Which of the following resides in caecum?** [UP 02]
   a. *Trichuris trichiuria* b. *A. Lumbricoides*
   c. *Strongyloides* d. *Ancylostoma*
   [Ref. Paniker 6/e, p 165]

8. **Which of the following is not a cestode?** [Kar 03]
   a. *D. latum* b. *T. saginata*
   c. *Schistosoma mansonia* d. *E. granulosus*
   [Ref. Paniker 6/e, p 139]

9. **Dracunculus medinensis is transmitted by:** [Bihar 03]
   a. Cyclops b. House fly
   c. Tick d. Flea
   [Ref. Ananthnarayan 7/e, p 213]

10. **Cysticercus cellulose seen in:** [Kolkata 04]
    a. *T. saginata* b. *T. solium*
    c. *D. latum* d. *S. haematobium*
    [Ref. Paniker 6/e, p 149]

11. ***Diphylobothrium latum* is causative organism of:** [SGPGI 04]
    a. Megaloblastic anemia
    b. Iron deficiency anemia
    c. Peptic ulcer
    d. None *[Ref. Paniker 6/e, p 142]*

12. **Hydatid cyst occurs most commonly in:** [DNB 04]
    a. Liver b. Lungs
    c. Brain d. Spleen
    [Ref. Paniker 6/e, p 153]

13. **One of the following transmitted through skin:** [UP 04]
    a. *Ascaris lumbricoides*
    b. *Trichuristrichiura*
    c. *Necator americanus*
    d. Strongyloides *[Ref. Paniker 6/e, p 159]*

14. **Which of the following is viviparous?** [SPGPGI 05]
    a. *Strongyloides stercoralis*
    b. *Trichinella spiralis*
    c. *Enterobius*
    d. *Ascaris* *[Ref. Paniker 6/e, p 159]*

15. **Visceral larva migrains is due to:** [DNB 05]
    a. *Ascariasis* b. Toxocaracanis
    c. Schistosomiasis d. Clonorchis buski
    e. Loa Loa *[Ref. Paniker 6/e, p 219]*

16. **The intermediate host for *T. saginata* is:** [DNB 05]
    a. Man b. Cow
    c. Dog d. Pig
    [Ref. Paniker 6/e, p 145]

17. **Neurocysticercosis is caused by:** [Jharkhand 05]
    a. *T. solium* b. *T. saginata*
    c. *D. latum* d. *Ascaris lumbricoides*
    [Ref. CMDT 08 p 1316]

Answers 1. d. Man and ... 2. b. Splenic ... 3. a. Man 4. b. *E. multilocularis* 5. b. *Enterobius* ...
6. d. Smooth ... 7. a. *Trichuris* ... 8. c. *Schistosoma* ... 9. a. Cyclops 10. b. *T.solium*
11. a. Megaloblastic ... 12. a. Liver 13. c and d 14. b. *Trichinella* ... 15. b. *Toxocaracanis*
16. b. Cow 17. a. T. solium

18. Autoinfection occur in: [UP 00]
a. *Strongyloides*
b. *Trichuris trichiura*
c. *Ancylostoma duodenale*
d. *Necator americanus* *[Ref. Paniker 6/e, p 171]*

19. One of the following microfilaria does not possess nuclei upto the tail tip: [Kar 0]
a. *Wuchereria bancrofti*
b. Loa loa
c. *Acanthochelonema perstans*
d. *Brugia malayi* *[Ref. Paniker 6/e, p 196]*

20. The following eggs have hexacanth embryos except: [Kar 00]
a. *Taenia solium*
b. *Taenia saginata*
c. *Chlonorchis nana*
d. *Hymenolepsis nana*

21. The larval form of taenia is referred to as: [Kar 01]
a. Cysticercus
b. Cysticercoid
c. Echinococcus
d. Conerus *[Ref. Paniker 6/e, p 148]*

22. Which worm is longest? [HPU 01]
a. *T. solium*
b. *T. saginata*
c. Hookworm
d. *A. lumbricoides* *[Ref. Paniker 6/e, p 144]*

23. Drug of choice in cerebral cysticercosis is: [JIPMER 01]
a. Piperazine
b. Pyrvinium
c. Thiabendazole
d. Mebendazol
e. None *[Ref. CMDT 08, p 1316]*

24. Katayama fever is caused by: [UP 01]
a. *F. hepatica*
b. *Clonorchis sinensis*
c. *S. hematobium*
d. *S. japonicum* *[Ref. Paniker 7/e, p 142]*

Katayama fever (Leukocytosis, eosinophilia, tender hepatomegaly) is more common in infection with S. japonicum

25. Ankylostoma duodenale commanly lives in:
a. Upper 1/3rd of duodenum [DNB 2011]
b. Lower 1/3rd of duodenum
c. Jejunum
d. Ileum *[Ref. Paniker 7/e p 182]*

26. Infective stage of hook worm is: [DNB 2014]
a. Trophozoite form
b. Filiform larva
c. Cyst
d. None *[Ref. Paniker 7/e p 104]*

27. Which of the following can be found in ocular muscles?
a. Trichinella b. Ascaris
c. Entrobius d. None *[Ref. Paniker 7/e p 168]*

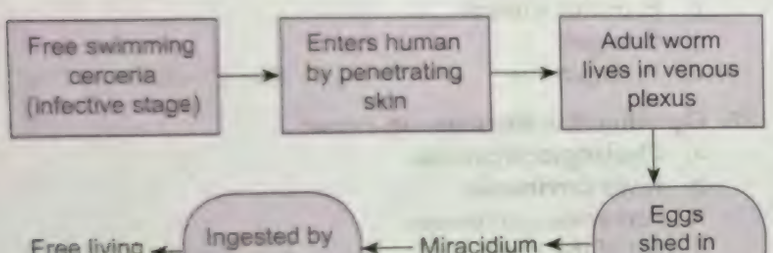
Answers 18. a. Strongyloides 19. a. Wuchereria ... 20. c. Chlonorchis ... 21. a. Cysticercus 22. b. T. saginata
23. None 24. d. S. japonicum 25. c. Jejunum 26. b. Filiform larva 27. a. Trichinella

NEET Pattern Questions

1. Katayama fever is caused by:
a. F. hepatica b. C. sinensis
c. S. haematobium d. A. lumbricoide
[Ref. Paniker 7/e, p 147]

Katayama fever on oriental schistosomiasis is caused by schistosoma japonicum.
- Katayama fever is characterized by eosinophilia, enlarged tender liver, and a palpable spleen. In the chronic stages several manifestations associated with portal hypertension are seen. Pathologically it is characterized by periportal fibrosis (clay pipe stem fibrosis)

2. Schistosomiasis is transmitted by:
a. Cyclops b. Fish
c. Snails d. Cattle
[Ref. Paniker 7/e, p 143]



3. Schistosomiasis is an exmple of:
a. Meta-zoonoses b. Cyclo-zoonoses
c. Direct-zoonoses d. Sporo-zoonoses

Metazoonosis: Disease transmitted by invertebrate host.

4. Cercariae are infective form of:
a. S. hematobium b. P. westermanii
c. F. hepatica d. T. solium
[Ref. Paniker 7/e, p 143]

5. Water host required for schistosomiasis:
a. Fish b. Cyclops
c. Snails d. Crabs
[Ref. Paniker 7/e, p 143]

6. Hydatid disease is caused by:
a. Echinococcus b. Tapeworm
c. Ascaris d. Clonorchis
[Ref. Paniker 7/e, p 127]

7. Wucheria bancrofti, true is:
a. Unsheathed b. Tail tip free from nuclei
c. Non-periodic d. All *[Ref. Paniker 7/e, p 205]*

8. Which is non lymphatic filariasis?
a. Loa loa b. Wucheria bancrofti
c. Brugia malai d. Brugia timori
[Ref. Paniker 7/e, p 205]

Loa Loa cause subcutaneous filariasis affecting subcutaneous tissue (calabor swelling) and conjunctivitis.

Lymphatic filariasis	Subcutaneous filariasis	Serous cavity filariasis
• Wuchereria bancrofti • Brugia malayi • Brugia timori	• Loa loa • Onchocerca vovulus • Mansonella streptocerca	• Mansonella perstans • Mansonella ozzard

9. True about diphyllobothrium:
a. Man is single host
b. Iron deficiency anemia is seen
c. Operculated egg is diagnostic
d. Fish is the definitive host
[Ref. Paniker 7/e, p 115-118]

Anaemia of Diphyllobothrium latum is due to vit B_{12} deficiency.

10. Unsegmented eggs are in which parasite?
a. Trichuris trichura b. Ancylostoma
c. Necator americanus d. Dracunculus
[Ref. Paniker 7/e, p 161]

Ascaris and Trichuris lay unsegmented eggs

11. Flame cells are seen in:
a. Protozoa b. Cestode
c. Nematodes d. None

Flame cells (solenocyte) are the excretory cell in cestodes and trematodes Arrangement and number of flame cell forms the basis for identification.

12. Rhabditiform larvae is seen in:
a. Tenia solium b. Strongyloides
c. D. latum d. Trichenella
[Ref. Paniker 7/e, p 173]

Answers 1. c. S. haematobium 2. c. Snails 3. a. Meta-zoonoses 4. a. S. hematobium 5. c. Snail
6. a. Echinococcus 7. b. Tail tip free from uncle 8. a. Loa loa
9. c. Operculated egg is diagnostic 10. a. Trichuris trichura 11. b. Cestode 12. b. Strongyloides

13. Which organism can be isolated from stool and sputum?
a. Paragnomus b. Fasciola
c. Chlornchis d. P. carini
[Ref. Paniker 7/e, p 157]

Helminth present in lung

Tremetode	Cestode	Nematodes
– Paragonimus westermani	– Echinoccocus granulosis	– Capillaria aerophila – Dicrofilaria imnitus

14. Larvae of Ascaris lumbricoidis most commonly causes:
a. Cardiac symptoms
b. Respiratory symptoms
c. Genitourinary symptoms
d. Cerebral symptoms *[Ref. Paniker 7/e, p 198]*

15. Ovoviviparous parasite which is associated with auto-infection:
a. Ancylostoma duodenale
b. Strongyloides stercoralis
c. Enterobius vermicularis
d. Ascaris *[Ref. Paniker 7/e, p 179]*

AutoInfection is seen in both Enterobius vermicularies and S.stercoralis. Enterobius is oviparous where as strongyloides is ovoviviparous.

16. Chlonorchis sinensis infection is due to ingestion of:
a. Fish b. Pork
c. Snail d. Beef
[Ref. Paniker 7/e, p 148]

17. Chlonorchis sinenensis is:
a. Tapeworm b. Roundworm
c. Threadworm d. Fluke *[Ref. Paniker 7/e, p 148]*

Clonorchis sinopsis is called Chinese liver fluke.

18. The following infection resembles malignancy:
a. Echinococcus granulosus
b. E.multilocularis
c. E.vogeli
d. E.oligarthus *[Ref. Paniker 7/e, p 134]*

Echinococcus multilocularis cause malignant hydatid disease, a benign condition that resembles clinically and prognosis wise to malignancy. Clinically it presents with multiple small cysts in both lobes of liver.
Most patient die of liver failure.

19. Skin penetration not seen in:
a. Taenia saginata
b. Nectator americanus
c. Ankylostoma duodenale
d. Strongyloides stercoralis *[Ref. Paniker 7/e, p 122]*

20. Larvae of Ascaris lumbricoidis most commonly causes:
a. Cardiac symptoms
b. Respiratory symptoms
c. Genitourinary symptoms
d. Cerebral symptoms *[Ref. Paniker 7/e, p 198]*

Clinical presentation of ascaris lumbricoides:
- Symptom due to larvae
  - Usually asymptomatic
  - Pathogenic effect of larval migration are due to allergic reaction and occur only when larval load is very heavy.
  - Pneumonitis is the commonest manifestation
- Symptom due to adult worm
  - Protein-energy malnutrition
  - Colicky abdominal pain
  - Intestinal obstruction
  - Acute biliary obstruction
  - Pancreatitis
  - Obstructive appendicitis
  - Liver abscess

21. Malignant hydatid cyst is caused by:
a. Echinococcus granulosus
b. E. multilocularis
c. E. vogeli
d. E. oligarthus *[Ref. Paniker 7/e, p 134]*

22. Opisthorchis sinensis can cause:
a. Cholangiocarcinoma
b. Liver carcinoma
c. Pancreatic carcinoma
d. All of the above *[Ref. Paniker 7/e, p 149]*

23. Cholangiocarcinoma is caused by:
a. Giardia lamblia
b. Clonorchis infestation
c. Paragonimus infestation
d. Ascaris infestation *[Ref. Paniker 7/e, p 149]*

24. Most common cause of cutaneous larva migrans:
a. Ankylostoma caninum
b. Toxocora canis
c. Toxocara cati
d. Ghathostoma *[Ref. Paniker 7/e, p 64]*

Most common cause of cutaneous larva migrans is non-human species of hook worm. (Ancylostoma braziliense and Ancylostoma-caninum)
Most common cause of visceral larva migrans is dog ascorid, Toxocara canis followed by cat ascarid T. cati

25. River blindness is caused by:
a. Onchocerca
b. Loa loa
c. Ascaris
d. B. malayi *[Ref. Paniker 7/e, p 217]*

Answers **13.a.** Paragnomus **14. b.** Respiratory... **15. b.** Strongyloides **16. a.** Fish **17. d.** Fluke
18. b. E.multilocularis **19. a.** Taenia saginata **20. b.** Respiratory symptoms **21. b.** E. multilocularis
22. a. Cholangiocarcinoma **23. b.** Clonorchis... **24. a.** Ankylostoma... **25. a.** Onchocerca

Section - B

UNIT – V Immunology

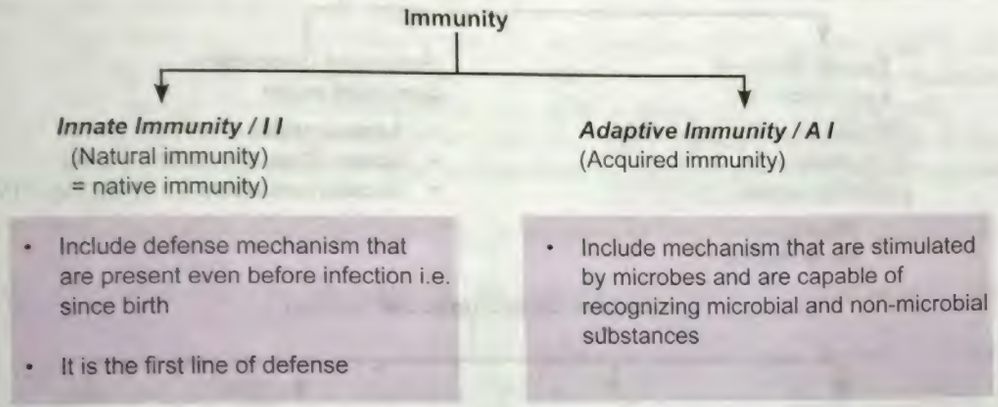
- Basics of Immune System
- Antigen and Antibody
- Hypersensitivity

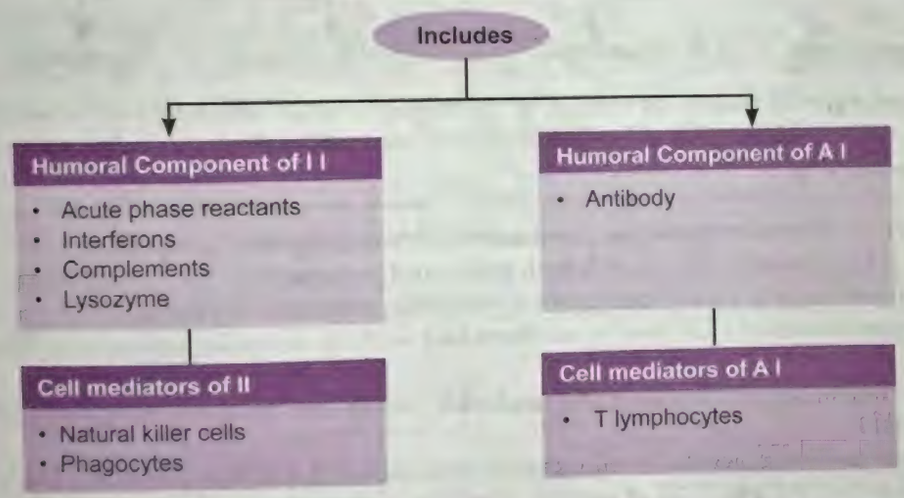
CHAPTER

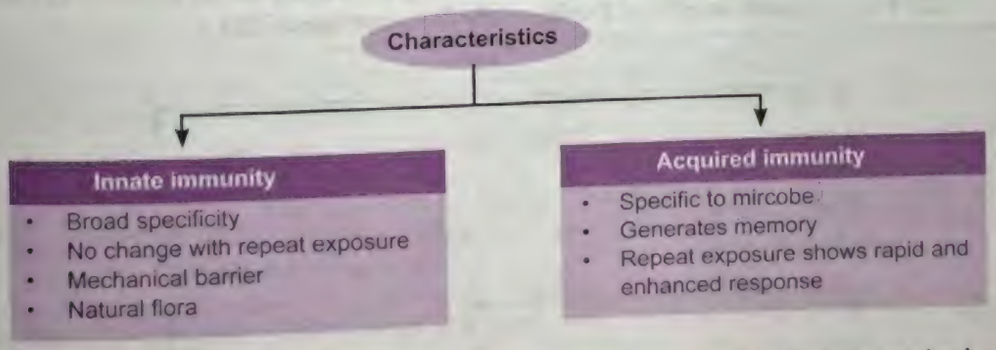
36

Basics of Immune System

CLASSIFICATION



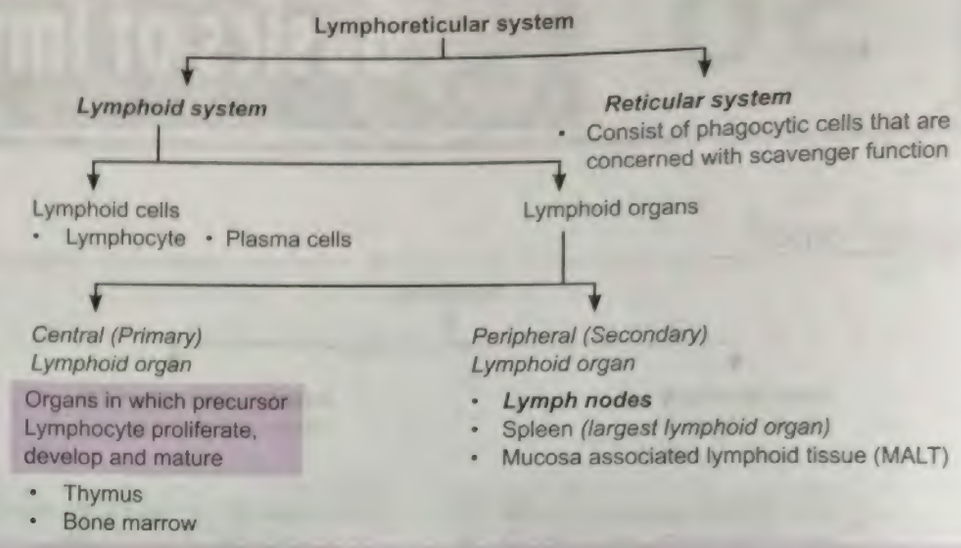


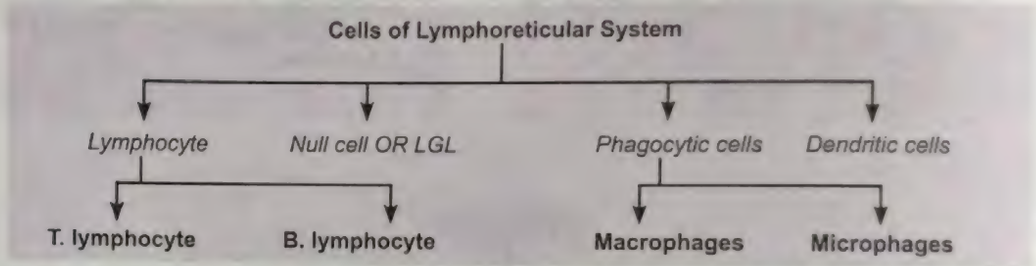


Innate immunity is the first line of defense and most potential pathogen are checked before they establish an overt infection. If these defenses are breached, the acquired immune system is called into play.

I

Lymphocytes
- B lymphocytes: Constitute 10-20% of peripheral lymphocyte. Provides humoral immunity.
- T lymphocyte: 60-70% of peripheral lymphocyte.





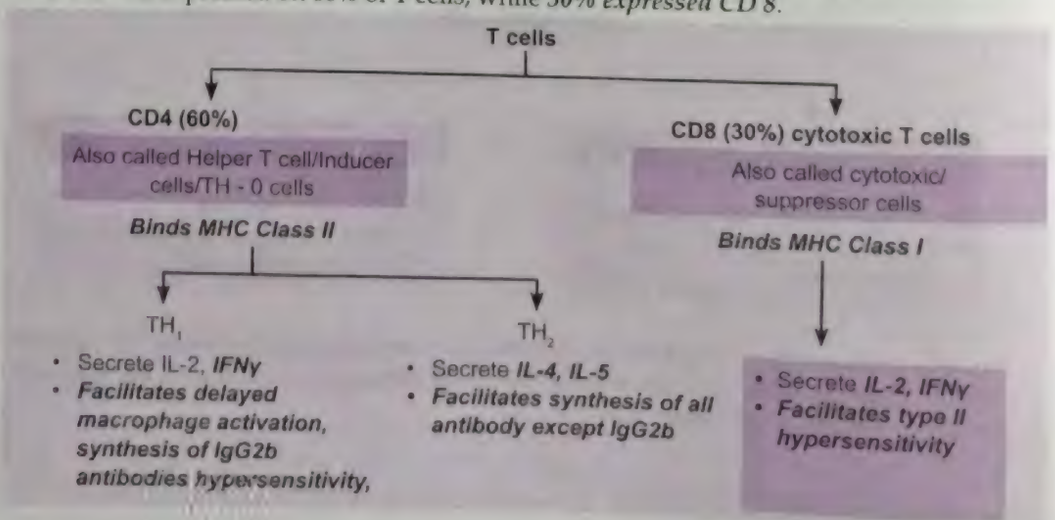
Lymphocyte - Human body contain 10^{12} lymphocyte out of which 10^{9} are renewed daily. Mature B and T cells before they encounter antigen are called **naive cells.**

T. LYMPHOCYTE

I

Naive cells:
Mature B or T cells who have not encountered antigen

- Thymus derived lymphocyte, constitute 60-70% of peripheral lymphocyte.
- Found in *paracortical* area of lymph nodes and periarteriolar sheaths of **spleen**.
- Antigen binds to TCR [T cell receptor] which is responsible for signal 1.
- Demonstration of TCR gene by southern blot analysis is a molecular *marker of T lineage cell.*
- All T-lymphocyte contain **CD-3 molecule** which are involved in transduction of signal 1.
- Other surface molecules or co-receptors include CD 2, CD 4 or CD 8, CD 11a, *CD 28 (Binds to B 7-1 and B 7-2 of antigen presenting cells and provide signal 2)*, CD 40.
- CD 4 is expressed on 60% of T cells, while *30% expressed CD 8.*



B. LYMPHOCYTE

- Develop from precursors in bone marrow.
- 10 - 20% of peripheral lymphocyte.
- Responsible for *humoral immunity.*
- Present in bone marrow, peripheral lymphoid tissue, e.g. lymph node (superficial cortex), spleen (white pulp), tonsils and extra lymphatic organs, e.g. GIT.
- In spleen and lymph node it *form lymphoid follicles.*
- Unlike T cell, it responds to *free Ag.*
- B cell act as *Antigen presenting* cells.
- Other molecules are complement receptor, Fc receptors, *CD 21 (receptor of EBV)*, CD 40 (essential for interaction of T and B cell which cause B cell *maturation* so mutation in *CD 40* ligand cause immunodeficiency called *X-linked hyper - IgM syndrome*).
- Membrane bound antibodies called IgM and IgD present on the surface of all nature naive B-cells and represent antigen-binding component of the B-cell receptor complex.
- RAG mediated rearrangement of Ig genes imparts B-cell receptor an unique antigen specificity.
- After stimulation by an antigen, B-cell develop into plasma cells that secrete antibodies.
- In addition to membrane Ig, the B-cell antigen receptor complex contains a heterodimer of two invariant protein called Igα and Igβ.
- First Ig class to appear on the B cell surface is Ig M. subsequently other classes may appear.

I

B. Lymphocytes:
- Act as APC
- Secrete Ig
- Responsible for humoral antibody

Differentiation of T and B cells		
	T cells	B cells
a. *Ag binding site*	Ag receptor (= TCR with CD. 3)	Surface Ig
b. *Fc receptor*	Absent	Present
c. *Complement receptor*	Absent	Present
d. *EAC rosette (C 3 receptor CR 2; EBV receptor)*	Absent	Present
e. *E/SRBC rosette (CD 2; measles receptor)*	+	–
f. *Microvilli on surface*	–	+
g. *Thymus specific Ag*	+	–
h. *Blast transformation*	Occurs by anti CD-3, Phytohemagglutinin Concanavalin	Occurs by anti-Ig Endotoxin *S. aureus* (cowan I strain) EBV

NULL CELL/LARGE GRANULAR LYMPHOCYTE (LGL)

- Constitute approximately 10–15% of peripheral blood lymphocyte.
- Differentiate and mature in bone marrow, lymph node, thymus, spleen.
- Morphologically NK cells are some what larger than small lymphocyte and contain abundant azurophilic granules.
- Functional activity is regulated by a balance between signals from activating and inhibitory receptors. NKG2D is the main activating receptor. CD–94 and killer cell Ig, like receptor are inhibitory receptor.
- ***Do not bear*** TCR or surface Ig and are non adherent, non-phagocytic.
- They together with macrophage *form innate immunity* in comparison *of adaptive* immunity *by lymphocytes.*
- **LGL express:**
  - Receptor for Fc portion of IgG (CD-16) which is used for ADCC **(antibody dependent cell mediated cytotoxicity)**.
  - Receptor for NCAM-I (CD 56).
- Many LGL express some T lineage markers particularly CD-8, CD-2.
- Usually CD-3 negative but subset of NK cell are *CD-3 positive* called NK/T cell.

- Some NK proliferate in the presence of *IL-2* called as LAK cells.
- **Target cell killing by NK cell is :**
  - Inversely related to target cell expression of MHC class I molecule.
  - So, it kills the cell that express little or no HLA class I molecule (provide immunosurveillance) such as ***virus infected cells, certain tumor cells and allogenic cells.***
  - Non-immune i.e. without previous sensitization, MHC unrestricted and non-antibody mediated.
  - It kill host cell infected with intracellular bacteria eg. Mycobacteria, TB, listeria monocytogens.
  - Not kill cells which express class I MHC (all normal nucleated cell express it).
- **Receptors**
  - *NK has two receptors:*
    a. Killer cell inhibitory receptors (KIRs) which recognized classic MHC-I and
    b. -CD 94/NKG- 2 receptor which recognized MHCIb or HLA - E.
- **NK cell secrete**
  - TNF α, GMCSF, IFN - γ, Cytolytic factors (perforin).
  - Also secrete IL-4 to recruit TH.2 T cell; IgG1, IgE.

I

ADCC is mediated by monocytes, neutrophils, eosinophils and NK-cells *...Robbins 8/e, p 202*

Remember:
- IFNγ favors differentiation of T_H 1 cell so NK cell can influence CD4 and B cell.
- NK cell is abnormal in HIV disease and hyporesponsive in Chediak-Higashi syndrome.

PHAGOCYTIC CELLS

It is of two types:

i. ***Mononuclear macrophages of blood and tissues :***
- Blood macrophages (monocytes) are ***largest*** of lymphoid cells.
- Tissue macrophages (histiocytes) are:
  - Microglia in CNS
  - Kupffer in liver
  - Alveolar macrophage in lung
  - Osteoclast in bone
  - Sinus histiocyte—spleen, lymph node.
- Half-life of *blood monocyte* is about *1 day* while life span of ***tissue macrophage*** is ***several months.***
- Most important cell in chronic inflammation in the form of activated macrophages, fusion giant cell and epitheloid cell of granulomatous lesion.
- When a monocyte reaches extravascular tissue, it undergoes transformation in to a larger phagocytic cell, macrophage. This macrophage activated by IFN - γ and other variety of stimuli secrete number of inflamatory mediators like IL-1, IL-6, INF - α.
- They are obligatory for induction of cell mediated immunity since they are required *to process and present antigen* to immuno competent T cell.
- Like other Ag presenting/processing cell, it has both class of MHC I and *II (more).*
- Also important for effector phase of humoral immunity.

ii. ***Microphages*** which are polymorphonuclear leucocytes of blood-neutrophil, eosinophil and basophil.

I

Phagocytic cells include
- Macrophages
- Microglia in CNS
- Kupffer cells of liver
- Osteoclast
- Sinus histiocytes.

DENDRITIC CELLS

- These are ***Antigen presenting cells (APC)*** to T cell during primary immune response.
- They are *bone marrow derived cells* of lineage different from macrophages and T or B cell.
- They possess MHC I and *II (more).*
- They have little or no phagocytic activity.
- *They are of following types:*

a. ***Interdigitating dendritic (= Dendritic) cells*** - **Most important APC for T-cells.** Found in lymphoid tissue and interstitium of non-lymphoid organs eg. heart and lung. Possess costimulatory molecules like B 7-1 and B 7-2.

I

Dendritic cells
- Main antigen presenting cells
- Include: Interdigitating dendritic cells
  - Langerhans cell
  - Follicular dendritic cells

Most potent antigen presenting cell for naive T cell (ideally CD - 4).

b. ***Langerhans cell*** - They process and present antigens which reach the dermis.

c. ***Follicular dendritic cells*** - Bear Fc receptor for IgG and receptors for C3b. Hence can trap antigen bound to antibodies. They are present in germinal centers of lymphoid follicles in spleen and lymph nodes present antigens to B-cell.

MAJOR HISTOCOMPATIBILITY COMPLEX (MHC)

- Located on short arm of chromosome six which codes for Histocompatibility (transplantation) antigen.
- Main function of Histocompatibility (MHC) molecule is to bind peptide fragments of foreign protein for presentation to appropriate antigen specific T cells. Human MHC antigen are synonymous with human leukocyte antigen (HLA), and the MHC complex of genes with the HLA complex.
- MHC gene products are classified as:

a. Class I antigens
- Are glycoprotein expressed on all nucleated cells and platelets.
- Cells with class I antigen presents antigen to CD - 8.
- It is the principal antigen involved in graft rejection and cell mediated cytolysis.
- Locus of class I gene - A, B, C.

b. Class II antigen
- Glycoprotein, restricted to antigen presenting cells (macrophage, dendritic cell, B cell) to CD - 4.
- Locus - D region.
- Responsible for graft versus host response and mixed leucocyte reaction (MLR).

c. Class III
- Soluble protein of complement system; (C_2 and C_4 of classical pathway; properdin factor B of alternative pathway); heat shock protein; TNF alpha and beta.

I

MHC
- Located on short arm of 6th chromosome
- MHC gene products:

a. Class I: Glycoprotein
b. Class II: Glycoprotein of antigen presenting cells
c. Class III: Soluble protein of complement.

HLA COMPLEX

HLA complex of genes are located on the **short arm of chromosome 6**. It consist of three separates cluster of genes:

1. HLA class 1 comprising A, B and C loci
2. Class II or the D region consisting of DR, DQ and DP loci.
3. Class III or the complement region containing genes for complement components C_2 and C_4.

HLA Molecules

HLA antigens are two-chain glycoprotein molecules anchored on the surface membrane of cells.

Class I molecules

- Consists of a heavy peptide chain (α chain) noncovalently linked to a much smaller peptide called β 2-microglobulin (β chain)
- The beta chain has a constant sequence which is coded by chromosomes 15.
- Alpha chain has got three domains (alpha 1, alpha 2 and alpha 3). The distal domain (alpha 1 and alpha 2) have highly variable amino acid sequences and are folded to form a groove.
- Protein antigen processed by macrophages or dendritic cells to form small peptides, are bound to this groove for presentation to CD8 T cells.
- HLA class 1 antigen are found on the surface of all nucleated cells.

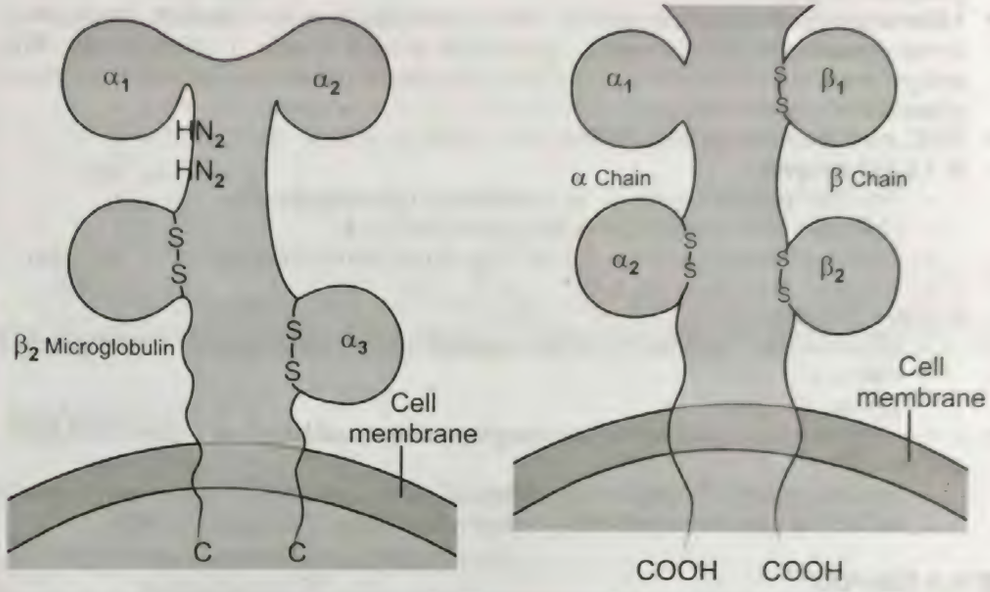
Class II molecules

- HLA class II antigen are found only on cells of the immune system—macrophages, dendritic cells, activated T cells, and particularly on B cells.
- Class II antigen are heterodimers, consisting of an alpha and a beta chain.
- Each chain has two domains, the proximal domain being the constant region and the distal the variable.
- The two distal domains (alpha 1, beta 1) constitute the antigen-binding site, for recognition by CD4 lymphocytes.

Class III molecules

- HLA class III molecules are heterogeneous. They include complement components linked to the formation of C3 convertases, heat shock proteins and tumor necrosis factors. They also display polymorphism.

Note: As you see above, human MHC complex of genes is synonymous with HLA.



HLA Class I and HLA Class II molecules

I
- Classic C3 convertase: **C4b2b**
- Classic C5 convertase: **C4b2b3b**

I
C5a is the most effective chemotaxis substance

COMPLEMENT SYSTEM

Consist of at least 30 chemically and immunologically distinct serum proteins.
Fixation of complement is not influenced by nature of antigens but only by class of Ig.

- IgM > IgG_3 > IgG_1 > IgG_2 – Classic pathway activators
- IgA_1, IgA_2, IgD, IgG_4 – Alternative pathway activators

Fractions: Complement is a complex of 9 different fractions

- ***Classic C3 convertase*** – C4b2b ***Classic C5 convertase*** – C4b2b3b
- ***Alternate C3 convertase*** – C3bBb ***Alternate C5 convertase*** – C3bBb3b
- Role of complement derived factors in inflammation:
  i. C3b and C3bi - act as opsonin so cause phagocytosis.
  ii. C3a + C5a [= Anaphylatoxin] - ↑ vascular permeability, vasodilation.
  iii. C5a - activate lipoxygenase pathway, chemotaxis, activation and adhesion.
  iv. C5b-9 (= Membrane attack complex) - Lysis of cell.
- Biosynthesis of complements:
  a. Intestinal epithelium: C1
  b. Macrophages: C2, C4
  c. Liver: C3, C6, C9
  d. Spleen: C5, C8

I
Complement function
Inflammation: C3a, C5a, C4a act as anaphylatoxin
C5a: Chemotaxin
Phagocytosis: C3b and C3bi,
Cell lysis: C5b–9

Clinical problems associated with genetic deficiencies of complement components	
Deficiency	**Syndrome**
CL inhibitor	Hereditary angioneurotic edema
Early components of classical pathway C1, C2, C4	SLE and othe collagen vascular diseases
C3 and its regulatory protein C3b inactivator	Severe recurrent pyogenic infections
C5 to C8	Bacteremia, mainly with Gram-negative diplococci, toxoplasmosis
C9	No particular diseases

INTERFERONS

- Originally discovered as factors produced by cells in response to viral infections.
- There are three main interferons:

α Interferon	β Interferon	γ Interferon
• Part of Innate immunity	• Part of innate immunity	• Part of acquired immunity
• Produced by peripheral blood mononuclear cells	• Produced predominanly by fibroblasts	• A lymphokine produce in response to a specific antigenic signal
• Type I interferon	• Type I interferon	• Type II interferon
• 18 different gene located on chromosome 9	• 1 gene located on chromosome 9	• 1 gene located on chromosome 9

- IFN α and IFN β share a common receptor whereas IFN γ binds to its own receptor.
- IFN α and IFN β are secreted in response to virus and certain intracellular bacteria. IFN γ is produced by antigen activated T-lymphocytes.
- All interferons upregulate class I MHC genes. IFN γ induces and increase the expression of MHC class II genes also.
- Interferons enhance the cytotoxcicity of macrophages, neutrophils, T-cells and NK cells. IFN γ produced by activated T-cells is capable of activating macrophages.
- Interferons induces a febrile response.

Multiple Choice Questions

1. **Adenosine deaminase deficiency is seen in the following:** [AI 05, 01]
   a. Common variable immunodeficiency
   b. Severe combined immunodeficiency
   c. Chronic granulomatous disease
   d. Nezelof syndrome

2. **A woman with infertility receives an ovary transplant from her sister who is an identical twin. What type of graft is it ?** [AI 05]
   a. Xenograft
   b. Autograft
   c. Allograft
   d. Isograft

3. **Which of the following statements is true about hapten?** [AI 04]
   a. It induces brisk immune response
   b. It needs carrier to induce immune response
   c. It is a T-independent antigen
   d. It has no association with MHC

4. **Neonatal thymectomy leads to:** [AI 02]
   a. Decreased size of germinal center
   b. Decreased size of paracortical areas
   c. Increased antibody marrow production by B cells
   d. Increased bone marrow production of lymphocytes

5. **IL-I produces:** [AI 02]
   a. T-lymphocyte activation
   b. Delayed wound healing
   c. Increased pain perception
   d. Decreased PMN release from bone marrow

6. **Regarding NK cells, false statement is:** [AI 01]
   a. It is activated by IL-2
   b. Expresses CD3 receptor
   c. It is a variant of large lymphocyte
   d. There is antibody induced proliferation of NK cells

7. **Which of the following best denotes classical complement pathway activation in immuno- inflammatory condition ?** [AIIMS 04]
   a. C2, C4 and C3 decreased
   b. C2 and C4 normal, C3 is decreased
   c. C3 normal and C2 C4 decreased
   d. C2, C4, C3 all are elevated

8. **Which of the following cell types are the most potent activator of T-cell ?** [AI 2012]
   a. Bell
   b. Follicular Dendritic Cells
   c. Mature dendritic cells
   d. Macrophages

9. **IL-2 is produced by:** [AI 97; 00; AIIMS 97]
   a. T cells b. B cells
   c. Monocytes d. Neutrophils

10. **All of the following are glycoproteins except:**
    a. Blood antigen b. Albumin [AI 97; 94]
    c. Immunoglobulin d. hCG

11. **Diagnosis of ABO incompatiability can be from all of the following except:** [AI 97]
    a. Sweat b. Saliva
    c. Semen d. CSF

12. **Helper cells belong to:** [AI 96]
    a. T cells b. Macrophages
    c. B cells d. Monocytes

13. **The type of receptors present on T cells are:** [AI 96]
    a. IgG b. IgD
    c. CD3 d. Prostaglandins

14. **What enhances multiplication of T cells in culture?**
    a. Phytohemagglutinin b. Chemotactic factor
    c. Leukotrienes d. Prostaglandins [AI 96]

15. **NK cells kill the viral infected cells due to:** [AIIMS 06]
    a. Increased expression of MHC class I molecules
    b. Decreased expression of MHC class I molecules
    c. Increased expression of MHC class II molecules
    d. Decreased expression of MHC class II molecules

16. **All of the following are part of innate immunity except:**
    a. Complement b. NK cells [AIIMS 05]
    c. Macrophages d. T cells

17. **Which is not a macrophage?** [AIIMS 97]
    a. Monocyte
    b. Microglia
    c. Kupffer cells
    d. Lymphocytes

18. **Common between B and T cells:** [PGI June 07]
    a. Origin from same cell lineage
    b. Site differentiation
    c. Antigenic marker
    d. Both humoral and cellular immunity
    e. Further differentiation seen

19. **Apart from B cells, and T cells, there is a 3rd distinct type of lymphocyte. This is:** [PGI 02]
    a. MHC cell
    b. NK cell
    c. Macrophage
    d. Neutrophil
    e. Microglia

20. All of these are antigens presenting cells except: [PGI 02]
a. T cells
b. B cells
c. Fibroblasts
d. Dendritic cells
e. Langerhans cells

21. Perforins are produced by: [PGI 02]
a. Cytotoxic T cells
b. Suppressor T cells
c. Memory helper T cells
d. Plasma cells
e. NK cells

22. IL-I is produced by: [PGI 00]
a. Macrophage
b. Helper T lymphocytes
c. B cells
d. Cytotoxic T-cells

23. Which complement component is involved in both classical and alternate pathway? [AI 2011]
a. C_1
b. C_2
c. C_3
d. All

24. Which of the following features is not shared between T cells and B cells? [AIIMS Nov 2012]
a. Positive selection during development
b. Class I MHC expression
c. Antigen specific receptors
d. All of the above

25. First chemical barrier encountered by microorganism for common exposed sites: [AIIMS Nov 2011]
a. Lysozyme
b. Acidic pH
c. Skin
d. Lactose

26. Active immunity can be induced by: [PGI May 2013]
a. Toxoids
b. Subclinical infection
c. Antitoxin
d. Immunoglobulins
e. Antigen exposure

27. True about passive immunity: [PGI May 2013]
a. Can not be given with active immunity
b. Last for 4-5 days only
c. It can be given before disease occurrence
d. Can be transferred by antibodies from another host
e. Takes long time to develop

28. The role played by Major Histocompatibility Complex-1 and 2 is to: [AIIMS 2013, 2014]
a. Transduce the signal to T cells following antigen recognition
b. Mediate immunogenic class switching
c. Present antigens for recognition by T cell antigen receptors
d. Enhance the secretion of cytokines

Explanations and References with Illustrative Answers

1. **Ans. (b) Severe combined immunodeficiency** *Ref. Ananthanarayan 8/e, p 158, 9/e, p 174; Harrison 19/e, p 2108*

Immunodeficiency — Primary immunodeficiency syndrome - genetically determined
— Secondary immunodeficiency syndrome, e.g. AIDS.

Classification of important Immunodeficiency syndrome	
Name of syndrome	*Defect*
i. Humoral immunodeficiency (B cell defects)	
a. X linked agammaglobulinemia:	Mutation in bruton tyrosine kinase
	Pre/pro B cell →✕→ B cell
b. Common variable immunodeficiency:	B cell →✕→ Plasma cells
c. Immunodeficiency with hyper IgM:	Mutation in CD40 ligand gene
ii. Cellular immunodeficiency (T cell defect)	
a. Thymic hypoplasia ***(Di George's syndrome):***	Failure of development of 3rd and 4th pharyngeal pouch (hypoplasia of thyroid and parathyroid also).
b. Chronic mucocutaneous candidiasis	
c. Purine nucleoside phosphorylase (PNP) deficiency	
iii. Combined immunodeficiencies (B and T cell defect)	
a. Cellular immunodeficiency with abnormal: Ig synthesis ***(Nezlof syndrome)***	Abnormal T cell maturation in thymus with normal, ↓ or ↑ Ig
b. Ataxia telengiectasia:	DNA repair defect
c. ***Wiskott-Aldrich syndrome:***	WASP gene mutation (secondary ↓ of T lymphocytes)
d. Severe combined immunodeficiency:	Variable defect

Severe Combined Immunodeficiency

- Includes syndromes with severe combined deficiency of both humoral and cell mediated immunity, inherited in **autosomal recessive mode.**
- Defects are at the level of early precursors of immunocompetent cells in the fetal liver and bone marrow.
- Some important types include:
  - **Swiss type agammaglobulinemia:** Basic defect is at the level of *lymphoid stem cell.*
  - **Reticular dysgenesis of de Waal:** Defect is at the level of *multipotent hemopoietic stem cell,* as a result of which there is total failure of myelopoiesis leading to leukopenia, thrombocytopenia and
  - **Adenosine deaminase deficiency:** Range of immunodeficiency varies from complete absence to mild abnormalities of B and T cell functions.

2. **Ans. (d) Isograft** *Ref. Ananthanarayan 8/e, p 178, 9/e, p 183*

Terminology of grafts		
Donor	Term	Synonyms
Self	Autograft	Autogenous or autogenic graft
Different individual, genetically identical with recipient. Identical twin or member of same inbred strain	Isograft	Isologous or syngeneic graft or syngraft
Genetically unrelated member of same species	Allograft	Allogeneic graft. Formerly called homograft
Different species	Xenograft	Xenogeneic. Formerly called heterograft

Type of graft

- *Orthotopic graft:* Graft applied in anatomically normal site, e.g. skin graft.
- *Heterotopic graft:* Graft applied in anatomicaly abnormal site, e.g. thyroid tissues transplanted in subcutaneous pocket.

3. Ans. (b) It needs carrier to induce immune response *Ref. Ananthanarayan 8/e, p 90, 9/e, p 87*

Haptens

- Substances that are incapable of inducing antibody formation by themselves but can react specifically with antibodies.
- Haptens become immunogenic on combining with a larger molecule or carrier.
- They are of two types:
  - Complex hapten: Precipitate with specific antibody
  - Simple hapten: Non precipitating.

4. Ans. (b) Decreased size of paracortical areas *Ref. Ananthanarayan 8/e, p 125, 9/e, p 130*

- Neonatal thymectomy leads to depletion of thymus dependent areas.
- *Peripheral lymphoid tissue is of two types:*

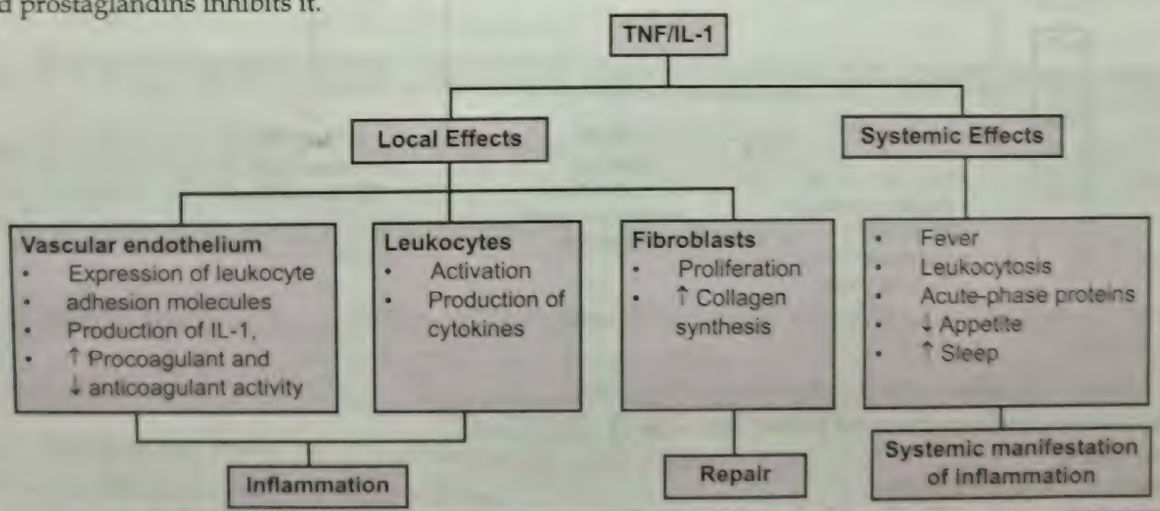
Features	Thymus dependent (contain T lymphocytes)	Bone marrow dependent (contain B lymphocytes)
Spleen	Lymphatic sheath surrounding the central arteriole known as Malpighian corpuscles in white pulp.	Perifollicular region, Germinal center, Mantle layer
Lymph node	Between cortical follicles and medullary cords there is ill-defined paracortical area.	The Corticle follicles and Medullary cords

- B cells also found in tonsils, extralymphoid organs such as GIT.

5. Ans. (a) T lymphocyte activation *Ref. Ananthanarayan 8/e, p 148, 9/e, p 154; Robbin's 7/e, p 82*

IL-1

- Formerly called as the leucocyte activating factor (LAF) and as the B-cell activating factor (BAF)
- Stable polypeptide; secreted by macrophages and monocytes but can be produced by most other nucleated cells also.
- Presence of antigens, toxins, injury and inflammation, stimulates its production, while cyclosporin A, corticosteroids and prostaglandins inhibits it.



Interleukins	Main source	Major function
IL-1 (α + β)	Macrophage	Proliferation and differentiation of T,B cells; pyrogenic; ↑ acute phase reactants; BM cell proliferation.
IL-2	TH1 cells	Growth and differentiation of T and B cells; cytotoxicity of T and NK cells
IL-3	T cells	Stimulation of hematopoietic progenitors.
IL-4	TH2 cells	Proliferation of B and cytotoxic T cells. ↑ IgG1 and IgE production; ↑ MHC class II and IgE receptor.
IL-5	TH2 cells	Proliferation of eosinophil; ↑ IgA and IgM production.
IL-6	TH1, macrophages,	Promote B cell differentiation, IgA production, Acute phase proteins.
IL-7	Spleen, BM stromal cells	B and T cell growth factor.

6. **Ans. (d) There is antibody induced proliferation of NK cells**

Ref. Harrison 17/e, p 2028, 18/e, p 2658; Ananthanarayan 9/e, p 137

NK cell activity is non-immune; MHC unrestricted; non-antibody mediated killer of target cells which are usually malignant cells types, transplanted foreign cells or virus infected cells.

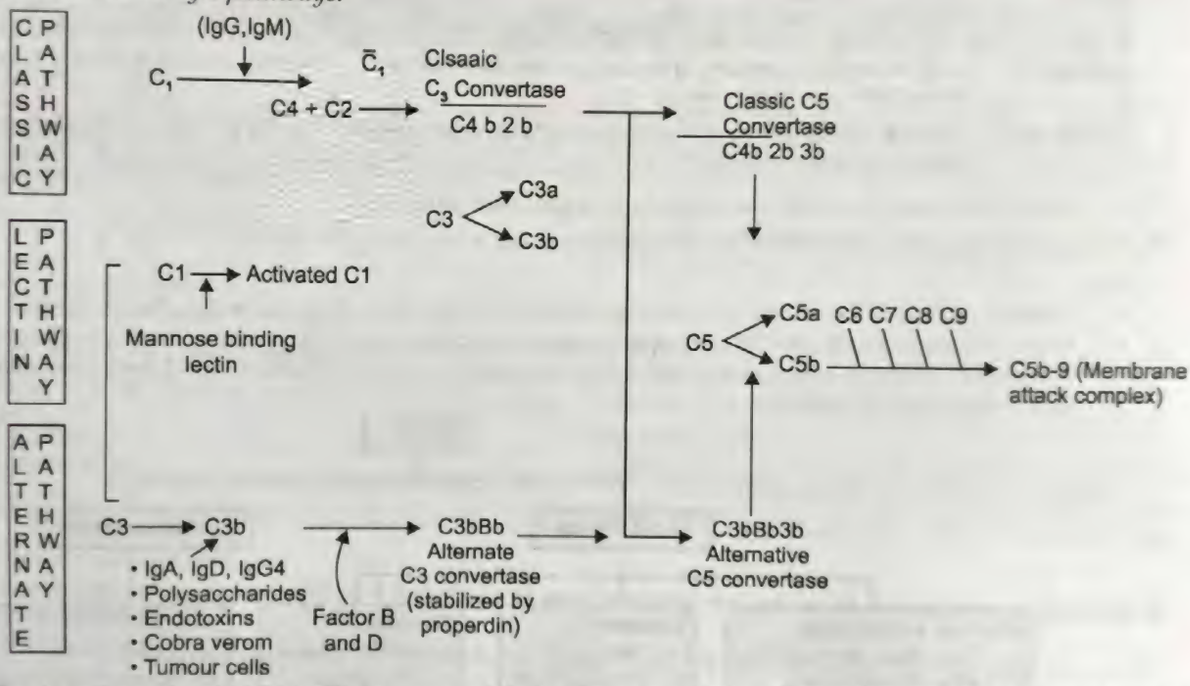
Thus NK cells play an important role in immune surveillance and destruction of malignant and virally infected host cells.

NK cells

- Accounts for 5-10% of peripheral blood lymphocytes. They are non adherent, non phagocytic cells with large azurophilic cytoplasmic granules.
- NK cells express surface receptors for the F_c portion of IgG (CD-16) amd for CD-56. Many NK cells express CD8, where as some NK cells (called as NK/T cells) express CD-3.
- NK cells proliferate in response to IL-2. NK cells arise in both bone marrow and thymic microenvironment.
- Three molecules of NK cells; NKp46; NK p30 and NKp44 are collectively referred as ***natural cytotoxicity receptors*** (NCRs) and mediate NK cell activation against target cells.

7. **Ans. (a) C2, C4, C3 decreased** *Ref. Jawetz 25/e, p 136; Harrison 17/e, p 2030, 18/e, p 2667; Robbins 7/e, p 66*

Complements are activated by 3 pathways.



- So, in Classic pathway level of C1 to C9 decreases while in Alternate pathway all complement levels decrease except C1, C2, C4.

8. **Ans. (c) Mature Dendritic cells** *Ref. Robins 8/e, p 192, Harrison 18/e, p 2657*

"Mature dendritic cells are the most potent activator of naive T-cell"

Dendritic Cells:

- Bone marrow derived cells
- There are two types of cells with dendritic morphology:
  - Interdigitating dendritic cells
  - Follicular dendritic cells
- ***Interdigitating dendritic cells*** or just dendritic cells are the most important antigen presenting cells for initiating primary immune response against protein antigens. This is due to following reasons:
  - These cells are located at the right place to capture antigens , i.e. under epithelia, in the interstitial of all tissue.
  - They express variety of receptors (including TLR, mannose) for capturing microbes.
  - In response to microbes dendritic cells express the same chemokine receptors as to naive T-cells.
  - They express high levels of MHC class II molecules as well as co-stimulatory molecules B.7-1 and B.7-2. Or in other words they possess all the machinery needed for presenting antigens to and activating CD4 + T cells.
- On the other hand ***follicular dendritic cells*** (*does not arise from bone marrow*) are present in the germinal centres of lymphoid follicles where they trap antigens bound to antibodies or complement. Follicular dendritic cells plays a role in ongoing immune response by presenting antigens to B-cells and selecting the B-cells that have the highest affinity for the antigen.

9. **Ans. (a) T-cells** *Ref. Ananthanarayan 8/e, p 148, 9/e, p 155*
Already explained

10. **Ans. (b) Albumin** *Ref. Harper 25/e, p 675; Lipincott 2/e, p 157*
Glycoprotein are proteins to which usually 2-10 oligosaccharides are covalently attached, e.g.

Glycoprotein		
• Ig	• Globular proteins except albumin	• Blood group antigen
• Mucin	• hCG, TSH	• Selectin
• Lectin	• HLA class I, II	• Secreted enzymes and proteins
• Glycophorin	• Transferrin, ceruloplasmin	• Collagen
• IFN β and γ		• Alkaline phosphatase

11. **Ans (d) CSF** *Ref. Reddy 5/e, p 378*
ABO group specific substances are found in high concentration in saliva, semen, vaginal secretion and gastric juice while in low concentration in sweat, tears and urine, so it is possible to determine blood group from an examination of these secretions.
So for this question it can be said that, they are present in all tissues except CSF.

12. **Ans. (a) T cells** *Ref. Ananthanarayan 8/e, p 130, 9/e, p 132-133*

Classification of T Cells			
Type of Cells	Surface Markers	Target Cells	Functions
Helper/inducer cell (TH)	CD4	MHC Class II restriction	Growth of T cells and Macrophages
Suppressor T cell (Ts)	CD8	MHC Class I	Down regulate the immune response
Cytotoxic/Cytolytic/Killer T Cell (Tc)	CD8	MHC Class I	Kill and lyse target cells carrying foreign antigen
Memory cells (Tm)	CD4 and CD8	MHC Class I	Provide memory and anamnestic responses

13. **Ans. (c) CD3** *Ref. Ananthanarayan 8/e, p 128, 9/e, p 133*

Surface markers	T cells	B cells
CD-3 receptor	+	–
Surface Ig	–	+
Thymus specific Ag	+	–
Ag receptor	+	–
Fc receptor	–	+
Complement receptor	–	+
Rosettes	SRBC or E. rosette (CD-2; measles receptor)	EAC rosette (C3 receptor; CR-2; EBV receptor)
Numerous microvilli	–	+

14. **Ans. (a) Phytohemagglutinin** *Ref. Ananthanarayan 8/e, p 127, 9/e, p 133*
Blast transformation or proliferation stimuli are:

Stimulus	T cell	B cell
Anti CD-3	+	–
Anti Ig.	–	+
PHA (Phytohemagglutinin)	+	–
Concanavalin A	+	–
Endotoxins	–	+
S-aureus (Cowan strain)	–	+
EB virus	–	+

Note: **(+)** means proliferation occurs and **(–)** means no proliferation

15. Ans. is a i.e. Increased expression of MHC class I molecules

Features of target cell killing by NK cell:

- Inversely related to target cell expression of MHC class I molecule.
  - So, it kills the cell that expresses little or no HLA class I molecule (provide immunosurveillance) such as ***virus infected cells, certain tumor cells and allogenic cells.***
- Non-immune, i.e. without previous sensitization, MHC unrestricted and non-antibody mediated.
- It kills host cell infected with intracellular bacteria, e.g. Mycobacteria, *TB. Listeria, monocytogens.*
- Do not kill cells which express class I MHC (all normal nucleated cells express it).

16. Ans. (d) T cells *Ref. Harrison 17/e, p 2021, 2031, 18/e, p 2651, 2668*

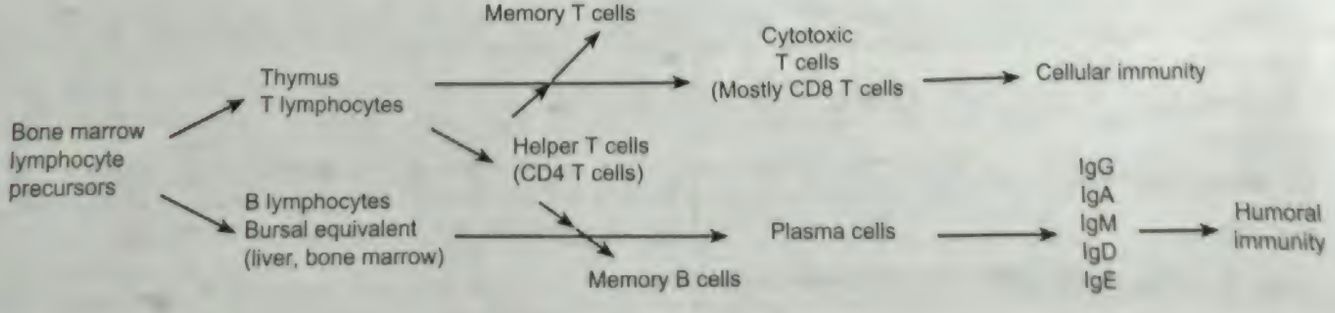
Components of the Adaptive Immune System	
Cellular	Thymus-derived (T) lymphocytes - T cell precursors in the thymus; naive mature T lymphocytes before antigen exposure; memory T lymphocytes after antigen contact; helper T lymphocytes for B and T cell responses; cytotoxic T lymphocytes that kill pathogen- infected target cells.
Humoral	Bone-marrow-derived (B) lymphocytes - B cell precursors in bone marrow; naive B cells prior to antigen recognition; memory B cells after antigen contact; plasma cells that secrete specific antibody.
Cytokines	Soluble proteins that direct focus and regulate specific T versus B lymphocyte immune responses.

Major Components of the Innate Immune System	
Pattern recognition receptors (PRR)	C type lectins, leucine-rich proteins, scavenger receptors, pentraxins, lipid transferases; integrins.
Antimicrobial peptides	α-Defensins, β-defensins, cathelin, protegrin, granulysin, histatin, secretory leukoprotease inhibitor, and probiotics.
Cells	Macrophages, dendritic cells, NK cells, NK-T cells, neutrophils, eosinophils, mast cells, basophils, and epithelial cells.
Complement components	Classic and alternative complement pathway, and proteins that bind complement components.
Cytokines	Autocrine, paracrine, endocrine cytokines that mediate host defence and inflammation, as well as recruit, direct, and regulate adaptive immune responses.

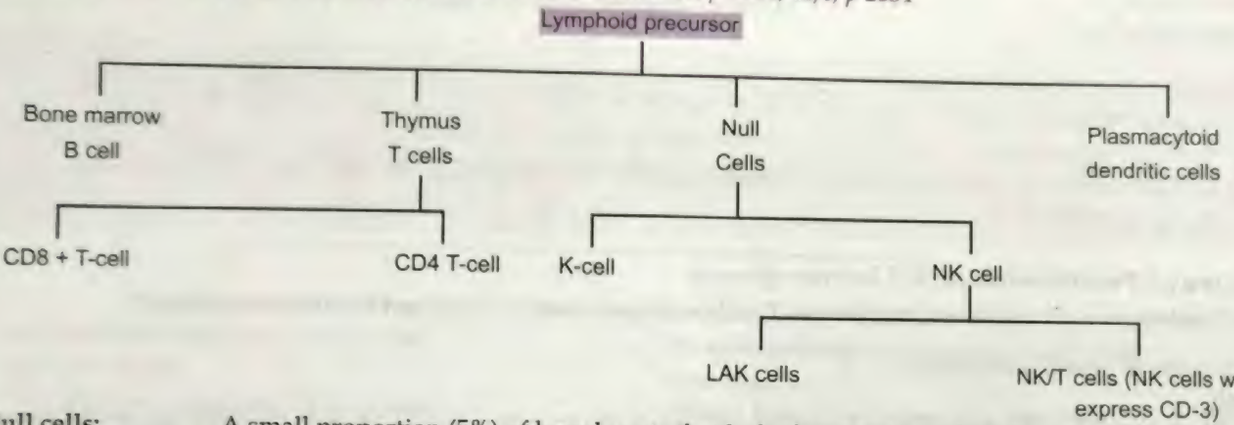
17. Ans. (d) Lymphocytes *Ref. Ananthnaryan 8/e, p 131, 9/e, p 137-138*

Macrophages	
Activation into	Differentiation into
Activated macrophages	Microglia (CNS)
Epitheloid cells	Kupffer (liver) cells
Giant cells (fusion type)	Alveolar macrophages (lung)
	Osteoclast (bone)
	Sinus histiocytes (spleen and lymph node)

18. Ans. (a) and (e) Origin from same cell lineage; further differentiation seen *Ref. Ganong 22/e, p 525*



19. Ans. is (b) NK cell *Ref. Dr Arora 3/e, p 156, p 127; Harrison 17/e, p 2024, 18/e, p 2651*



Null cells: A small proportion (5%) of lymphocyte that lack phenotypic markers B or T lymphocytes.

Killer cell: A sub-opulation of null cell which has surface receptor for Fc part of IgG. They are responsible for ADCC (antibody dependent cell mediated cytotoxicity).

Natural killer cells: These are large lymphocytes containing azurophilic granules in the cytoplasm. They are therefore cells known as large granular lymphocytes (LgL).

LAK (Lymphocyte activated killer) cells are NK lymphocytes that proliferate in response to high concentration of IL-2 and develop the ability to kills tumor cell more efficiently.

NK/T cells: These are CD3 + ve NK cells, that can recognize lipid molecules of intracellular bacteria when presented in the context of CD-1 molecules on antigen presenting cells.

These cells form an important defense against intracellular organism like histeria, mycobacterium.

20. Ans. (a) and (c) T cells and Fibroblast *Ref. Robbin's 8/e, p 192*

Antigen presenting cells are:

- Macrophages
- B cells
- Dendritic cells ***(most potent)***
- Activated T cells.

Antigen Presenting Cell

- An antigen presenting cell (APC) can be defined as any cell that expresses MHC or related molecule (eg CD1) that can bind antigenic components such as peptide, and can be recognized by one or another class of T-cell. T-cell can not recognize and therefore can't react to free antigen. T-cell can only see antigen which has been processed or presented by antigen presenting cells. Broadly on the basis of MHC class II expression APC can be divided in to:
  a. **Professional APC's :** APC's that express MHC II molecules, they are very efficient cells and include:
     - Macrophages
     - Dendritic cells
     - Certain activated epithelial cells
     - Few B-cells
  b. **Non professional APC:** They do not express MHC II molecule constitutively but only upon stimulation by certain cytokines. They include:
     - Fibroblast
     - Thymic epithelial cells
     - Thyroid epithelial cells
     - Glial cells
     - Pancreatic beta cells
     - Vascular endothelial cells

For examination purpose, only professional APC's are considered as APC.

21. Ans. is a i.e. Cytotoxic T cells *Ref. Robbin's 8/e, p 208*

Mechanism of CD-8 + T-cell Cytotoxicity

a. **Perforin-granzyme dependent killing:** Cytotoxic lymphocytes (CTL) secrete a complex consisting of perforin, granzymes and a protein called serglycin, which enters the target cells by endocytosis. In the cytoplasm of target cell perforin facilitates the release of granzymes from the complex. Granzymes in turn cleaves and activates caspases, which induce apoptosis of the target cells.

b. **Fas-Fas ligand dependent killing:** Activated CTL also express fas ligand which can bind to fas ligand on target cells and trigger apoptosis.

Note: T-cell mediated cytotoxicity is an important component in transplant rejection. β cell destruction in type I diabetes. They also play a role in reactions against virus infected cells.

22. Ans. (a) Macrophage *Ref. Ananthanarayan 8/e, p 148, 9/e, p 154; Robbin's 7/e, p 82*

Already explained in answer no. 5

23. Ans. is c i.e. C_3 *Ref. Jawetz 25/e, p 136*
Already explained

Remember: Biosynthesis of complement
- C_1 is synthesized in intestinal epithelium
- C_2, C_4 by macrophages
- C_5, C_8 in spleen
- C_3, C_6, C_9 in liver

24. Ans. is a i.e. Positive selection *Ref. Internet references*
B cell undergoes negative selection, whereas T cells undergoes both positive and negative selection.

Mechanism of central tolerance:
B cell:
- The recognition of antigens by the immature B-cells in the bone marrow is critical to the development of immunological tolerance to self. For proper immunological function it is essential B cell do not recognize self antigen and should recognize antigen derived from pathogens.
- In the bone marrow the immature B cells bearing surface IgM, if recognizes self molecule undergo negative selection. This self antigen induce loss of cells from the B cell repertoire is called as ***clonal deletion.*** Thus only those B cells that do not recognizes self molecules as antigen comes out of bone marrow.

T cells tolerance:
- T cells are selected for survival more rigorously than B cells. They undergo both positive and negative selection to produce T cells that recognizes self MHC but do not recognizes self peptides. T cell tolerance is induced in thymus.
  (i) ***Positive selection*** occurs in thymic cortex, if a maturing T cell is able to bind to a surface MHC molecule it is saved from program cells death; whereas cells fails to recognize MHC dies. Positive selection ensures that T cell recognize antigen in association with MHC molecule only.
  (ii) ***Negative selection*** occurs in cortex, cortico-medullary junction, and the medulla. Here MTEC (modullary thymic epithelial cells) signal self reactive T cells to die via apoptosis.

Other Options

Option b: Class I MHC are expressed by all nucleated cells and platelets. *Ref. Robbin's 8/e 190*

Option c: Both B cells and T cell possess antigen receptors. On B cells surface Ig acts as antigenic receptor whereas on T cell, TCR along with CD_3 acts as antigenic receptors. *Ref. AA 8/e 128, 9/e, p 133*

25. Ans. is b i.e. Acidic pH *Ref. Jawetz 25/e 124*
Physiological barriers at the portal of entry:

a. Skin:
- Skin provide the very efficient barrier against all sort of microbes, and only few bacteria can penetrate the skin.
- Sweat and sebaceous secretions by virtue of their acidic pH and certain chemical substances (fatty acids) have antimicrobial including antifungal properties.
- Lysozymes present on skin and other secretions also provide additional protection by lysing the microbes.

b. Mucous membrane:
- In the respiratory tract a film of mucous covers the surface. Bacteria tend to stick to this film. In addition there are lysozyme, and other antimicrobial substance.
- Saliva contains many enzyme.
- Gastric acidity kills almost all bacteria, ingested.

Remember: Innate immune system use pattern recognition receptor (like lipo-polysaccharide of Gr (–)ve bacteria, to sense the presence of pathogens.
Innate immune mechanism involves:
- Phagocytosis
- Inflammatory responses
- Interferons
- Alternate pathway of complement activation
- Fever
- Natural killer cells.

26. Ans. is a, b and e, i.e. Toxoids, Subclinical infection, Antigen exposure

Ref. Ananthanarayan 9/e, p 81-83

Acquired or Adaptive Immunity

Resistance that an individual acquires during life by recognizing and selectively eliminating specific foreign molecules

Active Immunity	Passive Immunity
• Induced by infections or by immunogens (Antigens, toxoid)	• Received passively, e.g. injection of readymade antibodies
• Provide effective protection for long period	• Effect is transient only immediate immunity
• Immunity effective only after lag period	• No memory
• Immunological memory present	• Subsequent dose is less effective
• Booster effect is there on subsequent exposure	• No negative phase
• Negative phase may occur	• Applicable in the immunodeficient
• Not applicable in the immunodeficients	• Anti rabies immunoglobulins are the example of passive immunity
• Live attenuated vaccines provide active immunity	

27. Ans. is c and d, i.e. It can be given before disease occurrence, Can be transferred by antibodies from another host

Ref. Ananthanarayan 9/e, p 81-83

The duration of passive immunity is approximately equal to the half life of the immunoglobulin injected, which varies from disease to disease.

Example: 7 days in case of antitetanus serum.

For full details See Previous Answer.

28. Ans. is c i.e. Present antigen

Ref. Ananthanarayan 9/e, p 142; Greenwood 18/e, p 106

Both Class I and Class II antigen are involved in presentation of processed antigen on microphage and other accessary cells to T-cell.

- Cytotoxic T-cell recognize Class I MHC antigen on target cells
- Helper T cell can accept antigens presented by macrophages/dendritic cells only when they bear some Class II MHC molecules
- MHC Class I molecules consist of two non covalently associated polypeptide chains A, B, C. Present on all human nucleated cells except neurons
- **MHC Class II Molecules:** are composed of two chains DP, DQ and DR. Found on immune cells (dendritis cells, macrophages, monocytes and activated T lymphocytes
- **MHC Class III genes:** They are grouped together in a region between D and B.

Chapter Review

1. **NK cells are:** [DNB 91]
   a. Activated macrophages
   b. Antibody-activated T cells
   c. Null cells activated by complement
   d. Derived from plasma cells
   e. Independent of antibody
   [Ref. Ananthanarayan 8/e, p 130, 9/e, p 137]

- Natural killer cells are one of the members of null cells, which lack features of both B lymphocyte & T lymphocyte. They have CD16 and CD-56 on their surface.
- NK cells possess spontaneous cytotoxicity towards various target cells, mainly malignant and virus infected cells.
- Their cytotoxicity is natural, i.e. not depends upon antibody or MHC restricted.

2. **T-cells functions are assessed by:** [AIIMS 92]
   a. Phagocytic index
   b. T-cell count
   c. Migration inhibition test
   d. Immunoglobulin index
   [Ref. Park 18/e, p 258]

3. **Interferon:** [PGI 93]
   a. Is species specific
   b. Reacts directly with virus particles to inactivate them
   c. Reacts with cells, and the affected cell then becomes resistant to a number of different viruses
   d. Constitutively produced at high levels in cells but requires an inducer for activity
   [Ref. Ananthanarayan, 8/e, p 452, 9/e p 448]

4. **T-cell multiplication is stimulated by:** [AI 93]
   a. Macrolin b. Heat
   c. Bovine serum d. Phytohemagglutin
   [Ref. Ananthanarayan 8/e, p 128, 9/e, p 133]

- T-cells undergo blast transformation on treatment with mitogens such as phytohemagglutinin, concanavalin A, while B-cells undergo similar trans-formation with bacterial endotoxins on E-B virus.

5. **Type of receptor present on T-cells are:** [AI 93]
   a. IgA b. IgG
   c. Prostaglandins d. CD4
   [Ref. Robbin's 7/e, p 197]

6. **Helper cells belong to:** [AI 93]
   a. Macrophages b. T-cells
   c. B-cells d. Monocytes
   [Ref. Ananthanarayan 8/e, p 130, 9/e, p 135]

7. **Lysozyme is present in the following secretions of the body except:** [Karnat 04]
   a. Lacrimal secretions
   b. CSF
   c. Saliva
   d. Respiratory tract secretions
   [Ref. Ananthanarayan 8/e, p 85, 9/e, p 180]

- Lysozyme is a thermolabile, low molecular weight basic protein which acts as neuraminidase. It is present in tissue fluids, and in nearly all secretions except CSF, sweat and urine. It acts by splitting certain polysaccharide components of the cell walls of susceptible bacteria.

8. **Virus infected cell is killed by:** [MP 05]
   a. Interferons b. Macrophages
   c. Neutrophils d. Autolysis
   e. None of the above
   [Ref. Ananthanarayan 8/e, p 447, 9/e, p 448]

- Virus infected cells are killed by NK cells, macrophages. Interferons themselves have no action but they act on other cells, rendering them refractory to virus infection.

9. **Interleukin - 7 produced by which one of the following?** [Kerla 00]
   a. Macrophages b. B cells
   c. T cells d. Dendritic cells
   e. Stromal cells
   [Ref. Ananthanarayan 8/e, p 148, 9/e, 154]

10. **Group B cell lymphocyte belongs to:** [UP 00]
    a. CD-19 b. CD-69
    c. CD-59 d. CD-68
    [Ref. Ananthanarayan, 8/e, p 128, 9/e, p 133]

11. **Defference between natural killer and cytotoxic cells is:** [UP 00]
    a. Interferons decreased natural killer acitivity
    b. NK has CD4
    c. Cytotoxic cell lyse IgG coated target cells
    d. NK contains azurophilic cytoplasmic granulocyte
    [Ref. Ananthanarayan 8/e, p 131, 9/e, p 137]

Answers 1. **e.** Independent 2. **c.** Migration 3. a and c 4. **d.** Phytohe ... 5. **d.** CD4
6. **b.** T-cells 7. **c.** Saliva 8. **b.** Macro... 9. **e.** Stromal ... 10. **a.** CD-19
11. **d.** NK ...

12. Which of the following cells is known as large granular lymphocyte (LGL)? [Karnat 01]
a. Plasma cells b. NK cells
c. T cells d. K cells
[Ref. Ananthanarayan 8/e, p 130, 9/e, p 137]

13. Which leukotriene is the adhesion factor for the neutrophill on the cell surface to attach to endothelium?
a. B4 b. C4 [Kerala 01]
c. D4 d. E4
[Ref. Robbin's 7/e, p 69 Table (2.4)]

14. Phagocytic cells elaborate: [JIPMER 01]
a. Prostaglandins b. Thromboxane
c. Leukotrienes d. All of the above
[Ref. Robbin's 7/e, p 68]

15. Runt disease is: [TN 02]
a. Graft rejection
b. Graft versus host reaction
c. Deficient T cell function
d. Complement deficiency
[Ref. Ananthanarayan 8/e, p 125, 9/e, p 130]

16. HLA III gene codes in graft rejection: [MP 03]
a. Immunological reaction in graft rejection
b. Complement
c. Graft virsus host reaction
d. Immunoglobulins
[Ref. Ananthanarayan 8/e, p 133, 9/e, p 140]

17. IL-2 is produced by: [SGPGI 04]
a. T cells (CD4 cells) b. B cells
c. Monocytes d. Neutrophils
[Ref. Ananthanarayan 8/e, p 148, 9/e, p 154]

18. DiGeorge syndrome is characterized by all except:
a. Congenital thymic hypoplasia [SGPGI 04]
b. Abnormal developmental of third and fourth pouches
c. Hypothyroidism
d. Hypocalcemic tetany
[Ref. Ananthanarayan 8/e, p 157, 9/e, p 173]

- **Thymic hypoplasia (Di George syndrome)**
  - It is a developmental defect involving the ***endodermal derivatives of the third and fourth pharyngeal pouches***, leading to aplasia on hypoplasia of the thymus and parathyroid gland.
  - It is nonhereditary and is supposed to be due to intrauterine infection
  - It is associated with tetralogy of Fallot.
  - Neonatal tetany is present
  - There is defect in cell mediated immunity and humoral immunity is largely unaffected.

19. Phagocytic function is assessed by: [MP 06]
a. Proliferative response to mitogen
b. Reduction of NBT (Nitroblue tetrazolium test)
c. Serum immunoglobulin assay
d. Skin test with purified protein derivative
[Ref. Harrison 17/e, p 381]

20. Which of the following cells is known as large granular lymphocyte ? [Kar 01]
a. Plasma cells
b. NK cells
c. K cells
d. T cells *[Ref. Ananthanarayan 8/e, p 130, 9/e, p 137]*

21. Transplantation of the hosts own tissue is known as:
a. Isograft b. Allograft [Kar 01]
c. Xenograft d. Autograft
[Ref. Learn it]

22. Pro-inflammatory cytokines include all except: [Kar 01]
a. Interleukin 1
b. Interleukin 2
c. Interleukin 6
d. TNF-alfa *[Ref. Harrison 17/e, p 1156]*

23. Chemoattractant is: [Jharkhand 03]
a. C5a b. C1
c. C3 d. C2
[Ref. Greenwood 18/e, p 117]

24. Complement C5-C9 predispose to which infection?
a. Meningococci [Jharkhand 03]
b. Pneumococcal
c. Pseudomonas
d. All *[Ref. Harrison 17/e, p 911]*

25. Which of the following acts as a chemoattractant?
a. C3a b. C3b [DNB 2012]
c. C5a d. LTB4
[Ref. Robbin's 8/e, 50]

CHEMOATTRACTANTS

Endogenous	Exogenous
– Cytokines (IL-8) – Components of complement system (C5a) – Arachidonic acid metabolites (leukotriene B4)	– Bacterial products that possess N-formyl methionine terminal amino acid and some lipids

26. Fever is caused by: [DNB 2013]
a. IL 3 b. IL 6
c. IL 5 d. IL 9
[Ref. Robbin's, 8/e, p 55]

Answers 12. b. NK cells 13. a. B4 14. a and c 15. c. Deficient ... 16. b. Complement
17. a. T cells ... 18. c. Hypothy ... 19. b and c 20. b. NK cells 21. d. Autograft
22. b. Interleukin 2 23. a. C5a 24. a. Menigococi 25. d. LTB4 26. b. IL 6

NEET Pattern Questions

1. Complement formed in liver:
a. C2, C4 b. C3, C6, C9
c. C5, C8 d. C1

2. C-3 convertase in alternate complement pathway:
a. C4b2a b. C3b
c. C3bBb d. C3a
[Ref. Ananthanarayan, 9/e, p 124]

3. Activation of classical complement pathway:
a. IgA b. IgG
c. IgM d. IgD
[Ref. Ananthanarayan, 9/e, p 121]

First step of activation of complement pathway is binding of C1 to the antigen-antibody complex. One molecule of IgM or two molecules, IgG can initiate this process

4. Opsonization takes place through:
a. C3a b. C3b
c. C5a d. C5b

5. Center of complement pathway:
a. C3 b. C1
c. C5 d. C2
[Ref. Ananthanarayan, 9/e, p 121]

Once C3 activation occurs, the subsequent steps are common in classic, alternate and lectin pathways

6. Gene components of HLA class I includes:
a. A, B, C b. DR
c. DQ d. DP
[Ref. Ananthanarayan, 9/e, p 140]

HLA class	Product
Class I	A, B, C loci
Class II	DR, DQ, DP
Class III	C2, C4, TNF α and β, properdin factor B

7. HLA complex is on chromosome:
a. 6 b. 7
c. 8 d. 9
[Ref. Ananthanarayan, 9/e, p 140]

8. Chediak Higashi syndrome, defect is:
a. Fusion of lysosome b. T-cells
c. B-cells d. Complement
[Ref. Harrison, 18/e, p 478]

In chediak-Higashi syndrome (Autosomal recessive) there is reduced chemotaxis and phagolysosome fusion. Blood picture show giant primary granules in neutrophils and other granule bearing cells. Clinically these patients present with decreased pigmentation of eye, skin and hair.

9. Hereditary angioneurotic edema is due to:
a. Deficiency of C inhibitor
b. Deficiency of NADPH oxidase
c. Deficiency of MPO
d. Deficiency of properdin
[Ref. Ananthanarayan, 9/e, p 116]

10. SCID which is true:
a. Adenosine deaminase deficiency
b. Decreased circulating lymphocytes
c. NADPH oxidase deficiency
d. C1 esterase deficiency *[Ref. Ananthanarayan, 9/e, p 179]*

11. Nucleotidase deficiency:
a. Humoral immunity deficiency
b. Acquired immunity deficiency
c. SCIDs
d. Cell mediated immunity deficiency
[Ref. Ananthanarayan, 9/e, p 124]

Ecto-5 nucleotidase deficiency is associated by B cell defect

12. All are true about innate immunity except:
a. Non-specific
b. First line of defence
c. Not affected by genetic make up
d. Includes complement
[Ref. Ananthanarayan, 9/e, p 79]

13. Which of the following T Cell independent Antigen:
a. T-cell b. B-cell
c. Macrophages d. CD8+T cells

14. Not true about innate immunity:
a. Not influenced by hormones
b. Dependent on genetic constitution
c. Identical twins have same degree of resistance
d. Not influenced by exposure to antigen
[Ref. Ananthanarayan, 9/e, p 79]

Endocrine disorders such as diabetes mellitus, hypothyroidism and adrenal dysfunction are associated with increased susceptiblity to infection

Answers 1. b. C3, C6, C9 2. c. C3bBb 3. b, c. IgG, IgM 4. b. C3b 5. a. C3
6. a. A, B, C 7. a. 6 8. a. Fusion of... 9. a. Deficiency of... 10. a. Adenosine...
11. a. Humoral 12. c. Not affected by ... 13. b. B-cell 14. a. Not influenced...

15. When transfer factor is given as treatment results in:
a. Natural active immunity
b. Artificial active immunity
c. Artificial passive immunity
d. Adoptive immunity
[Ref. Ananthanarayan, 8/e, p 89]

16. Most potent chemoattracatant is:
a. Leukotriene B4 b. Leukotriene C4
c. Leukotriene D4 d. Leukotriene E4
[Ref. Robbins, 8/e, p 57]

17. T cells in lymph node are present in:
a. Paracortical area b. Mantle layer
c. Medullary cords d. Cortical follicles
[Ref. Ananthanarayan, 9/e, p 131]

18. Which cells cause rosette formation with sheep RBCs:
a. T-Cells b. NK cells
c. Monocytes d. B cells

TH1 cells promote cell mediate immunity by producing IL-2, which activate macrophages and T-cells. TH-2 cells produce cytokines IL4, 5 and 6 which stimulate B cells to form antibodies.
TH1t cells produce cytokine IL17 and promote inflammation.

19. Cellular immunity is induced by:
a. NK-Cells b. Dendritic-cells
c. TH1-cells d. TH2-cells
[Ref. Ananthanarayan, 9/e, p 135]

- T cells bind to sheep erythrocytes, forming rosettes (SRBC or E rosette) by the CD2 antigen. B. cells do not.
- B cells bind to sheep erythrocytes coated with antibody and complement, forming EAC rosettes, due to the presence of a C3 receptor (CR2) on the B cell surface. CR2 also acts as a receptor for the Epstein-Barr virus. T cells do not possess this.

20. In cell lysis by compliments:
a. They activate cyclaise
b. Inhibits elongation factor p
c. Destruction of cell wall
d. Increased permeability of cell membrane
[Ref. Ananthanarayan, 9/e, p 122]

21. True about interferon is:
a. Host protein
b. Viral protein
c. Inactivated by nucleases
d. Virus specific *[Ref. Ananthanarayan, 9/e, p 155]*

22. Chronic granulomatous disorder is due to defect in:
a. B-cell b. NADPH oxidase
c. IgA d. T-cell
[Ref. Ananthanarayan, 9/e, p 178]

The mechanism of complement mediated cytolysis is the production of holes, approx. 100 Å diameter on cell membrane, which disrupts the osmotic integrity of membrane and lysis.

23. Anaphylatoxin in complement system:
a. C_{3b} b. C_{5-9}
c. C_{5a} d. C_{1-3}
[Ref. Jawetz, 27/e, p 141]

24. Most chemotactic property is with:
a. C3a b. C5a
c. C5-9 d. C3b
[Ref. Jawetz, 27/e, p 141]

25. Membrane attack complex (MAC) in complement system is:
a. C_{3b} b. C_{1-3}
c. C_{5-9} d. C_{2-4}
[Ref. Jawetz, 27/e, p 142]

26. Complement components are:
a. Lipids
b. Proteins
c. Lipoproteins
d. Polysaccharide
[Ref. Ananthanarayan, 9/e, p 121]

27. Which is not true about macrophages:
a. Activation by IFN-γ
b. Major cells in chronic inflammation
c. M_2 type involved in inflammation
d. Phagocytic cells
[Ref. Robbins, 8/e, p 70, Greenwood, 18/e, p 121]

28. Natural killer cell is:
a. MHC restricted
b. Antibody dependent
c. Null cells
d. B-lymphocytes
[Ref. Robbins, 8/e, p 165]

29. Which does not stimulate active immunity:
a. Subclinical infection
b. Clinical infection
c. Vaccination
d. Transplacental antibody in newborn
[Ref. Ananthanarayan, 9/e, p 83]

Answers 15. d. Adoptive... 16. a. Leukotriene B4 17. a. Paracortical area 18. a. T-Cells 19. c. TH1-cells
20. c. Destruction of... 21. a. Host protein 22. b. NADPH oxidase 23. c. C_{5a} 24. b. C5a
25. c. C_{5-9} 26. b. Proteins 27. c. M_2 type... 28 c. Null cells
29. d. Transplacental antibody in newborn

30. Complement deficiency has not been associated with:
a. SLE
b. PNH
c. Hereditary angioedema
d. Membranous nephritis

[Ref. Ananthanarayan, 9/e, p 125]

31. Primary T-cell deficiency is:
a. Ecto-5 nucleotidase deficiency
b. Common variable immunodeficiency
c. DiGeorge syndrome
d. Wiskott-Aldrich syndrome

[Ref. Ananthanarayan, 9/e, p 172]

32. MHC class I :-

34. Functions of complement include all except:
a. Chemotaxis
b. Opsonization
c. Cell lysis
d. Antigen presentation

[Ref. Robbins, 8/e, p 64]

35. Total number of complement factors in complement system:
a. 7

CHAPTER

37

Antigen and Antibody

I

- **Haptens:** Nonimmune, but react with antibody
- **Epitope:** Smallest unit of antigenicity
- **Paratope:** Area on antibody that bind epitope.
- **Idiotopes:** Specific antigen determinant on paratope.

ANTIGENS

Substance that can provoke the production of antibody.

Determinants of Antigenicity

- Molecular size (< 5000 are non-antigenic)
- Chemical nature (usually protein and polysaccharide are more antigenic than lipid and nucleic acid)
- Susceptibility to tissue enzymes
- Foreignness
- Antigen specificity
- Species specificity
- Isospecificity
- Autospecificity (except lens protein and sperm)
- Organ specificity
- Heterogenetic/heterophile specificity (Forssman antigen; Weil-Felix reaction in typhus fever, Paul-Bunnel test in infectious mononucleosis; Cold agglutinin test in primary atypical pneumonia).

Haptens – Non-immunogenic but has immunological reactivity, i.e. incapable of inducing antibody formation but can react with antibodies.
They become immunogenic on combining with larger molecule carrier.

Hapten is of two types:

i. Simple haptens are non-precipitating.
ii. Complex haptens are precipitating, since they have two or more antibody combining sites.

Epitope or antigenic determinant

- It is ***smallest unit of antigenicity*** (small area on the antigen) which is capable of sensitising an immunocyte and of reacting with its complementary site on specific antibody or T cell receptor.
- *T* cell identify ***linear or sequential*** epitope, whereas *B* cell identify ***conformational*** epitope.

Paratope

- It is combining area of the antibody molecule, corresponding to the epitope.
- Epitope and paratope determine **specificity** which is hallmark of immunological reaction.
- Bacteria/virus may contain antigen mosaic (different epitopes) while same epitope on different antigen may present causing antigenic cross-reaction.
- Specific antigen determinants on paratope are called *IDIOTOPES.*

ANTIBODIES

Plasma		
Fractionation of serum proteins by NH_4SO_4		
Soluble albumins	***Insoluble globulin***	
	Water soluble (pseudoglobulins)	Insoluble (Euglobulin)

- ***Most*** of the human antibody is euglobulin and are usually gammaglobulin (but equine antitoxin is beta or alpha globulins).

- All antibodies are Ig but all Ig may not be antibodies because Ig not only includes antibody globulins but also includes abnormal proteins of myeloma macroglobulinemia, cryoglobulinemia and naturally occurring subunits of Ig.
- Ig constitutes 20-25% of total serum proteins.

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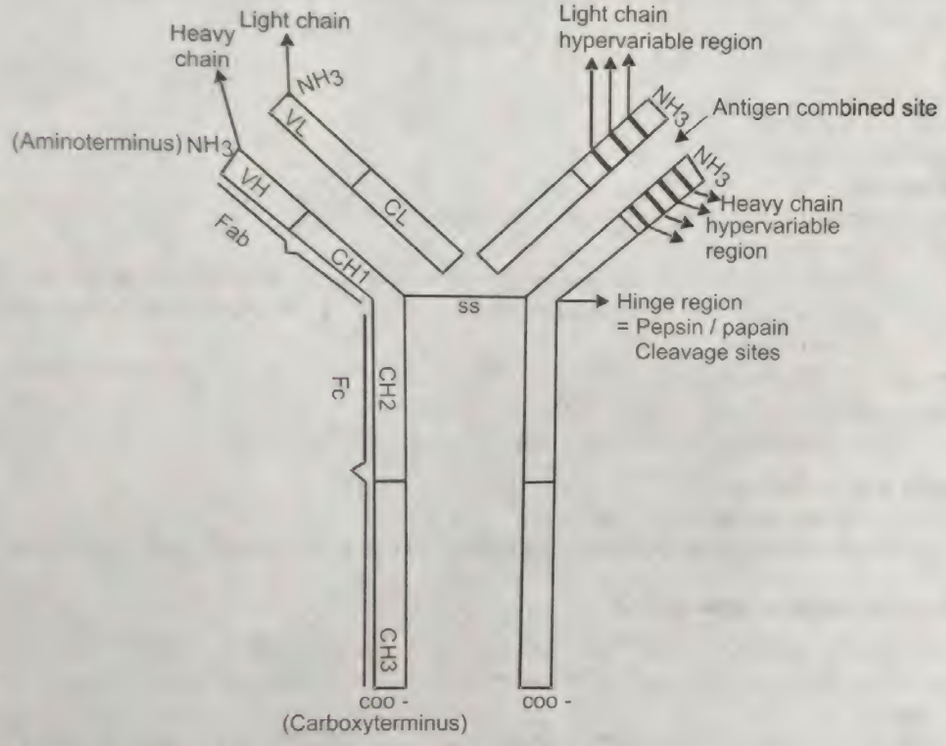
Antibodies:
- Globulin in nature
- Contains 2 heavy chain and 2 light chain
- Heavy chain has 4 highly variable region.
- Light chain has 3 highly variable region.

Structure of Ig

- **VL** = Variable domain of light chain.
- **CL** = Constant domain of light chain.
- **VH** = Variable domain of heavy chain.
- **CH** = Constant domain of heavy chain.
- **S-S** = Disulphide bond.



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Antibodies:
- Glycoproteins
- Present in serum and body fluids.

- ***Constant region*** = Carboxyterminus = Fc = [contains only heavy chain] which determines Ig biological properties.
- ***Variable region*** = Aminoterminus = Fab = Antigen binding region [= contains both Heavy and Light chains] which determines immunological specificity of antibody molecule.
- Ig (glycoprotein) consists of two pairs of polypeptide chains **(2H and 2L).**
- H = heavy chain has molecular weight 50,000.
  - H chain are structurally and antigenically distinct for each class and are designated by Greek letter γ, α, μ, δ, ε corresponding to Ig class IgG, IgA, IgM, IgD, IgE. respectively.
  - H chain consist of 1 variable (VH) and three domains in constant region (CH_1, CH_2, CH_3).
- L = Light chain with molecular weight of 25,000.
  - L chain is similar in all classes of Ig.
  - 2 types of L chain are kappa (κ) and lambda (λ).
  - 1 molecule of Ig may have either kappa or lambda chains but never both.
  - Kappa and Lambda occur in ratio of about κ : λ = 2 : 1.
  - L chain consists of 1 variable (V_L) and 1 constant domain (C_L).
- *Highly variable zones* (3 in L and 4 in H chain) are known as *Hypervariable regions* or *Hot spots.* They are involved in the formation of antigen-binding sites. Sites on the hypervariable

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Heaviest Ig	: IgM
Lightest Ig	: IgA
Heat labile	: IgE
Most abundant	: IgG in serum IgM in plasma

regions which make actual contact with the epitope are called *complementarity determining regions* or *CDRs*.

- Fd piece - is portion of H chain present in Fab fragment.
- Immunoglobin isotype (G, M, A, D, E) is determined on the basis of Ig heavy chain present.

Immunoglobulin classes

It has following properties:

- *Sedimentation coefficient* - ***max*** IgM
- *Molecular weight* - ***max*** IgM ***min*** - IgG
- *Serum concentration, Half life in days, Daily production (mg/kg)* - G > A > M > D > E
- *Intravascular distribution (%)* - ***max*** IgM, ***min*** IgA
- *Carbohydrate (%)* ***max*** IgE
- *Complement fixation:* Classical - IgM > IgG
  Alternative - IgA, IgD, IgG
- *Placental transport* - only IgG
- *Present in milk* - IgG and IgA
- *Selective secretion by seromucinous gland* - IgA
- *Heat labile* - **only** IgE
- J chain - IgA and IgM

Properties of various immunoglobulin classes

	IgG	IgA	IgM	IgD	IgE
1. Molecular weight in kDa	150	160,385*	900	180	190
2. Sedimentation coefficient	7	7,11	19	7	8
3. Carbohydrate content (%)	3	8	12	13	12
4. Heavy chain	$\gamma_1, \gamma_2, \gamma_3, \gamma_4$	α_1, α_2,	μ	δ	ε
5. Light chain	κ, or λ	κ, or λ	κ, or λ	κ, or λ	κ, or λ
6. Serum concentration (mg/ml)	12	2	1.2	0.03	0.00004
7. Half-life (days)	21	6	5	3	2
8. Complement binding	Classical pathway	Alternative pathway	Classical pathway	None	None
9. Binding to tissue	Heterologous	None	None	None	Homologous
10. Secretion from serous membranes	No	Yes*	No	No	Yes
11. Placental Passage	Yes	No	No	No	No
12. Heat stability (56°C)	Yes	Yes	Yes	Yes	No

Immunoglobulin classes

i. **IgG**
- Most abundant immunoglobulin of serum, making up 75% of total
- General purpose antibody, enhances phagocytosis by opsonization.
- It has four subclasses G_1 (65%), > G_2 (23%), > G_3 (8%) > G_4 (4%)
- IgG decrease from birth to reach minimum levels by 3rd month.

ii. **IgA**
- Occur in two forms - ***Serum IgA*** (monomer) and ***secretory IgA***, i.e. SIgA (dimer joined by J chain present on respiratory/intestinal mucosa and in secretions).
- J chain is also produced by plasma cells but secretory piece/secretory component of SIgA is not produced by lymphoid cells but by mucosal or glandular epithelial cells. Secretory component protects IgA from denaturation.
- Has two classes IgA_1 (primarily found in serum) and IgA_2 (found in secretions)
- Most abundent antibody in secretions

iii. **IgM**
- Effective valency is five.
- **Earliest Ig** to be synthesized by fetus (begin at 20 week of age) is IgM.
- At 20th weeks Peyer's patches and lymphoid cells in spleen, and lymph nodes are developed so fetus has IgM, IgD, IgG (transplacentally) but not IgA and IgE.
- IgM detection is useful in diagnosis of congenital infection.
- Monomeric IgM is major antibody receptor on surface of B lymphocytes for antigen recognition.
- IgM synthesis is five times more costly for a cell in comparison to IgG, this is the reason for IgM to IgG switch.

iv. **IgD**
- Resemble IgG structurally and also serve as recognition receptor for antigen.

v. **IgE**
- Mostly extravascular and exhibits Homocytotrophism.
- Chiefly produced in the lining of intestinal and respiratory tract.
- It mediates Reaginic hypersensitivity and Prausnitz-Küstner (PK) reaction.

Remember: *IgG protect body fluids, IgA body surfaces and IgM the blood stream.*

Types of Antibody in various conditions:

IgM
- Biological false positive Ab in syphilis
- Rheumatoid factor
- Ab against ABO
- Ab to typhoid O Ag (endotoxin).

IgG
- Ab against Rh factor (Anti Rh D)
- (LATS) long acting thyroid stimulator Ab in Graves'
- AutoAb in SLE, GB Syndrome
- Reagin Ab in syphilis (Lupus anticoagulant, anticardiolipin).

Abnormal Ig

i. ***Bence Jones Protein (BJP)***
- Monoclonal Ig consists of light chain found typically in multiple myeloma.
- Identified in urine by its characteristic property of coagulation when heated to 50°C but redissolving at 70°C.

ii. ***Myeloma (M) protein***
- Monoclonal Ab seen in multiple myeloma (IgG, IgA, IgD, IgE) and Waldenstrom's macroglobulinemia (IgM).

iii. ***F_c parts of Ig heavy chain***
- ↑ in Heavy chain disease.

iv. ***Cryoglobulinemia***
- Formation of gel or precipitate on cooling the serum, which redissolves on warming.
- Most cryoglobulin consist of either IgG, IgM or mixed precipitates.

IMMUNOGLOBULIN SPECIFICITIES

- It is of great importance in immumolegy. The antigenic determinants on immuglobulin molecule fall in to three main category.
- **Isotype:** Isotype variations refers to genetic variations and differences in the constant region of the heavy chain of the Ig class or subclass within a species. Within a species each normal individuals will express all isotypes in the serum whereas different species inherit different constant region and therefore express differet isotypes.
- **Allotype :** It refers to multiple allele that exists for some of the genes and which lead to subtle amino acid difference in some but not all members of species, e.g. Allotype have been characterized for all IgG subclass. Each of these allotypic determinants represent differences in one to four amino acid that are encoded by different alleles.
- **Idiotype:** These determinants arise from the sequence of the heavy and light chain variables region. The sum of all idiotopes on an Ig molecule constitute idiotype. This results due to the variability in amino acid sequence in variable region. Antibodies against the idiotypic portion of an antibody molecule are called anti-idiotype antibodies.

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IgG : Main antibody synthesized in re-exposure
IgM: Antibody synthesized in first exposure
IgA: Antibody found in secretions
IgE: Antibody involved in activating mast cells and basophills

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IgM Vs IgG

IgM is 500-1000 times more effective than IgG in opsonisation.
100 times more effective in bactericidal action
20 times more effective in bacterial agglutination

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IgG protects body fluid
IgA protects body surface
IgM protects blood stream
IgE produce hypersensitivity
IgD is the recognition molecule on the surface of B lymphocyte

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Serological Reactions:
- ***Precipitation:*** Soluble Antigen + Antibody in presence of electrolyte
- ***Agglutination:*** Particulate antigen + antibody in presence of electrolyte. More sensitive include, tube and slide agglutination

ANTIGEN – ANTIBODY REACTIONS

Ag-Ab reaction is reversible, occur ***at surface*** and there is no denaturation of Ag or Ab during reaction. Reactions occur in three stages:

i. ***Primary stage:***
Ag-Ab combined by weaker intermolecular forces such as van der Waals, ionic bond and H_2 binding rather than by firmer covalent bonding; without any visible effects.

ii. ***Secondary stage:***
Usually present but not always. It leads to demonstrable events such as precipitation, agglutination, lysis of cells etc.

iii. ***Tertiary stage (reaction):***
Leads to tissue damage e.g. allergy and other immunological diseases.

Serological reactions

Comparative efficiency of Ig in different serological reactions:

Reaction	IgG	IgM	IgA
• *Neutralization (N)*	More effective	Less effective	Variable
• *Precipitation (P)*	Strong	Weak	Variable
• *Classical complement fixation (C)*	Strong	Strongest	Negative
• *Immunohemolysis (I)* and *Bactericidal (opsonization) (O)*	Less effective	More effective	Moderate
• *Agglutination (A)*	Weak	Strong	Moderate
• *Lysis (L)*	Weak	Strong	Negative

Mnemonic for strong reaction in IgG = *G*-N-P (Neutralization, Precipitation).
Mnemonic for strong reaction in IgM = CO-*M*-ALI (Classical Complement fixation, Opsonization, Agglutination, lysis, Immunohemolysis).

Precipitation Reaction

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Prozone phenomenon is clinically seen in secondary syphillis

When ***soluble antigen*** combines with its antibody in presence of electrolytes, it forms *insoluble precipitates/floccules.*

- This reaction show "zone phenomenon" ***(also in agglutination)*** either in the form of prozone (***antibody*** excess) or postzone (***antigen*** excess).
- It occurs in zone of equivalence due to lattice formation ***(also in agglutination)***.
- It is very ***sensitive*** in antigen detection (but relatively less sensitive for antibody detection).
- It is of following types:
  a. ***Ring test*** – Simplest type, e.g. Ascoli's thermoprecipitin test and streptococcal lancefield grouping.
  b. ***Slide test*** – VDRL test of syphilis
  c. ***Tube test*** – Kahn test of syphilis
  d. ***Immunodiffusion (ppt in gel)*** – e.g. Elek test for toxigenicity in diphtheria (double diffusion).
  e. ***Electroimmunodiffusion*** – e.g. rocket electrophoresis for quantitative estimation of antigen.

Immunodifusion

- Possess advantage of visible and stable reaction. It includes:
  - Single diffusion in one dimension (Oudin procedure)
  - Double diffusion in one dimension (Oakle-Fulthorpe procedure)
  - Single diffusion in two dimension (Radial immunodiffusion)
  - Double diffusion in two dimension (Ouchter lony procedure)

Agglutination Reaction

- When *particulate antigen* combines with its antibody in the presence of electrolyte at suitable pH and temperature. The particles gets clumped or agglutinated.

- It is more sensitive than precipitation for antibody detection. Occur in presence of electrolytes.
- Incomplete or monovalent antibodies (usually Ab are bivalent) do not cause agglutination, though they combine with the antigen. They also act as blocking Ab since they inhibit agglutination by complete Ab.
- Agglutination is of following types:
  a. ***Slide agglutination*** – used for blood grouping and cross matching.
  b. ***Tube agglutination*** – e.g. Widal test, Brucellosis, Weil-Felix reaction, Paul Bunnel test, cold agglutination and *Streptococcus* MG test.
  c. ***Antiglobulin (Coombs) test*** – used for detecting incomplete Ab of brucellosis; anti-Rh Ab.
  d. ***Passive agglutination test*** – used to detect Ab by adsorbing soluble Ag on carrier particles so precipitation reaction converts into agglutination test which are *more convenient* and *more sensitive*. e.g. Rose Waller test, test detecting RA factor by using amboceptor.
  e. ***Latex agglutination test (latex fixation test)*** – for detection of ASO, CRP, RA factor, HCG; Streptozyme test.
  f. ***Reversed passive agglutination*** –Estimation of antigen by adsorbing antibody to carrier particles.

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- Complement fixation test: ***Wassermann reaction***
- Neutralization test: **Schick test**

Complement Fixation Test (CFT)

- Antigen may be soluble or particulate.
- Source of complement is guinea pig serum, e.g. Wassermann reaction; coaglutinating complement adsorption test using horse complement; immuno adherence of *V. cholera* and *T. pallidum*; Immobilization test of *T. pallidum*; Cytolytic or cytocidal test.

Neutralisation Tests

- Includes virus neutralization test ***(plaque inhibition test)***, Toxin neutralization ***(Schick, antistreptolysin O)*** test; Nagler's reaction

Radio-Immunoassay (RIA)

- *MC* labels used are radio-isotopes and enzymes.
- It measures analytes upto picogram (10^{-12}g) quantities.
- Used for quantitation of hormones, drugs, tumour markers, IgE and viral antigen.

Enzyme Immunoassay (EIA)

- Measures enzyme labelled antigen, hapten or antibody.
- It may be homogenous or heterogenous.
- Major type of heterogenous EIA is ELISA which involves the use of immunosorbent - an absorbing material specific for one of components of reaction, the antigen or antibody, e.g. ELISA for detection of ***Rotavirus in feces or HIV antibody in serum.***

Lattice Hypothesis

- Explains precipitation and agglutination.
- Multivalent antigen combines with bivalent antibodies in varying proportion
- Precipitation and agglutination occurs at zone of equivalence.
- In zone of antibody excess (prozone phenomenon) or antigen excess (post zone) lattice do not form.

Multiple Choice Questions

1. **The serum concentration of which of the following human IgG subclasses is maximum ?** [AI 05]
   a. IgG1 b. IgG2
   c. IgG3 d. IgG4

2. **The earliest immunoglobulin to be synthesized by the fetus is:** [AI 03]
   a. IgA b. IgG
   c. IgE d. IgM

3. **All of the following forces are involved in antigen antibody reaction *except*:** [AI 98, AIIMS 96]
   a. Van der Waals forces b. Electrostatic bond
   c. Hydrogen bond d. Covalent bond

4. **Which of the following statements concerning immunoglobulins is wrong?** [AI 97]
   a. IgM does not cross placenta
   b. IgE is decreased in parasitic infection
   c. IgM is increased in primary response
   d. Fetal infection is characterized by increase in IgG

5. **Which precipitates at 50°C-60°C but disappears on heating ?** [AI 96]
   a. Heavy chain b. Light chain
   c. Both d. None of the above

6. **Bence Jones proteins are best described as:** [AI 96]
   a. m chains
   b. g chains
   c. Kappa and lambda chains
   d. Fibrin split products

7. **Prozone phenomenon is due to:** [AI 96, PGI 03]
   a. Antigen excess b. Antibody excess
   c. False +ve reaction d. False -ve reaction

8. **A single immunoglobulin molecule contains:** [AI 95]
   a. 1 light chain, 1 heavy chain
   b. 2 heavy chains, 1 light chain
   c. 2 light chains, 2 heavy chain
   d. 2 light chains, 1 heavy chain

9. **The following methods of diagnosis utilize labeled antibodies *except*:** [AIIMS 05]
   a. ELISA
   b. Hemagglutination inhibition test
   c. Radioimmunoassay
   d. Immunofluorescence

10. **Which of the following is class specific antigenic determinants of an Ig ?** [AIIMS 04]
    a. L-chain\
    b. H-chain
    c. J-chain
    d. Variable region

11. **In a 5-year-old boy who has history of recurrent pyogenic infections by bacteria with polysaccharide-rich capsules, which of the following investigations should be done?** [AI 2012, AIIMS May 2012]
    a. IgA deficiency b. IgG_1 deficiency
    c. IgG_2 deficiency d. IgA and IgG_2 deficiency

12. **The most avidly complement fixing antibody is:** [AIIMS 02]
    a. IgA b. IgG
    c. IgM d. IgE

13. **All of the following statements about carbohydrate antigens are true *except*:** [AI 2011]
    a. Good memory response
    b. Poor immunogenicity
    c. T-cell independent immunity
    d. Polyclonal response

14. **Which of the following is true ?** [AIIMS 96]
    a. Paul Bunnell test is used to diagnose measles
    b. Rose Waller test is a complement fixation test
    c. Indirect hemagglutination test is less sensitive than gel diffusion test
    d. Antigen, antibody reaction cannot occur in absence of electrolytes

15. **Vaccination is based on the principle of:** [AI 2012]
    a. Agglutination
    b. Phagocytosis
    c. Immunological memory
    d. Clonal detection

16. **The reaction between antibody and soluble antigen is demonstrated by:** [AIIMS 96]
    a. Agglutination b. Precipitation
    c. Complement fixation d. Hemagglutination test

17. **C-reactive protein is:** [AIIMS 96]
    a. An antibody as a result of pneumococcal infection
    b. Derived from pneumococci
    c. Detected by precipitation reaction
    d. Increased in pneumococcal infection

18. **Antibody diversity is due to:** [PGI Dec. 07]
    a. Gene rearrangement
    b. Gene translocation c. Antigenic variation
    d. CD40 molecules e. Mutation

19. **MHC class III genes encode:** [PGI 06]
    a. Complement component C3
    b. Tumor necrosis factor - alpha
    c. Tumor necrosis factor - beta
    d. Interleukin 2
    e. Beta 2 microglobulin

20. Acute phase reactants (APR) in acute inflammation are: [PGI 03]
a. Albumin
b. Fibrinogen
c. Haptoglobulin
d. Gammaglobulin

21. Antigen binding site on antibody is: [PGI 02]
a. Hinge region
b. Constant region
c. Variable region
d. Hypervariable region
e. Idiotype region

22. IgE is secreted by: [PGI 02]
a. Mast cell
b. Basophils
c. Eosinophils
d. Plasma cells
e. Neutrophils

23. Classic complement is activated by: [PGI 02]
a. IgG
b. IgA
c. IgM
d. IgE
e. IgD

24. The secretory component of IgA molecule is: [PGI 01]
a. Formed by epithelial cell of lining mucosa
b. Formed by plasma cell
c. Formed by epithelial cell and plasma cell
d. Secreted by bone marrow

25. True of the following is/are: [PGI 01]
a. IgA crosses placenta
b. Half-life of IgG is 23 days
c. IgD is heat stable
d. IgE has highest carbohydrate content
e. IgG induces leukotrienes release during inflammation

26. Which of the following immunoglobulins can cross placenta? [PGI 01]
a. IgA
b. IgM
c. IgG
d. IgD

27. C.reactive proteins are: [PGI 00]
a. Alpha-globulin
b. Beta-1 globulin
c. Alpha-2 globulin
d. Non-specific inflammatory protein

28. Which is not heterophile agglutination test? [PGI 2001]
a. Weil-Felix test
b. Widal test
c. Paul-Bunnel, test
d. Streptococcus MG

29. True about secondary immune response is: [PGI 98, 96]
a. Long latent period
b. Usually of low titer
c. Antibodies appear in short time
d. Persist for long

30. True about interferon is: [PGI 98]
a. It is a synthetic antiviral agent
b. Inhibits viral replication in cells
c. Is specific for particular virus
d. None

31. Which of the following is very difficult to induce antibody? [PGI 97]
a. Polysaccharide
b. Protein
c. Antigens
d. Repeated infection

32. True regarding clonal selection [PGI 2008]
a. Specific
b. Secondary response is rapid
c. Variable region of Ig is involved in Ab production

33. Which of the following is true about isotypic variation? [AIIMS 09]
a. Subtle amino acid changes due to allelic difference
b. Changes in a heavy and light chain in variable region
c. Changes in heavy and light chain in constant region responsible for class and subtype
d. These are areas in antigen that bind specifically to antibody

34. Superantigens true statement is [AIIMS May 10]
a. They bind to the cleft of the MHC
b. Needs to be processed before presentation
c. They are presented by APC's to T-cells
d. Directly attached to lateral aspect of TCR β chain

35. Antigen antibody precipitation is maximally seen in which of the following: [AIIMS May 10]
a. Excess of antibody
b. Excess of antigen
c. Equivalent of antibody and antigen
d. Antigen-Hapten interaction

36. Antigen-antibody complexes are detected by:
a. Western blot [PGI 09]
b. Southern blot
c. Northern blot
d. ELISA

37. Which portion of MHC 1 complex forms the component of antigen presenting part: [AIIMS 2011]
a. Between alpha1-beta 2 microglobulin
b. Distal part of alpha chain
c. Proximal part of alpha chain
d. Between a_s and b_2 microglobulin

38. C in C-reactive protein stands for: [AI 2011]
a. Capsular polysaccharide of S. pneumoniae
b. Concanavalin-A
c. Calretinin
d. Cellular

39. Ova albumin antigen was injected into a rabbit. What antibody will it produce initially? [AI 2011]
a. IgG
b. IgM
c. IgE
d. IgD

40. Role of adjuvant in vaccine is/are: [PGI Nov 2011]
a. Stimulation of Toll-like receptors
b. Activate B-lymphocyte only
c. Increase both adaptive and innate immune response
d. Activate both B and T lymphocyte
e. Ensure prolonged delivery of antigen

41. **Synthesis of an immunoglobulin in membrane bound or secretory form is determined by:** [AIIMS: May 2012]
    a. One turn to two turn joining rule
    b. Class switching
    c. Differential RNA processing
    d. Alletic exclusion

42. **Immunoglobulin bound on the surface of bacteria mediates phagocytosis by:** [AIIMS: Nov 2011]
    a. C3b and Fc
    b. Receptor mediated endocytosis
    c. Oxidase action
    d. Lysosomal burst

43. **Heterophile agglutination is/are used in all test *except*:**
    a. Widal test [PGI May 2013]
    b. Weil-Felix reaction
    c. Paul-Bunnel test
    d. ELISA
    e. Cold agglutination test

44. **True about antibody:** [PGI May 2013]
    a. IgM is produced in primary response
    b. IgD protects mucosa
    c. IgE is main antibody in secondary response
    d. IgG is main antibody in secondary response
    e. IgA protects body surface

45. **Antibodies present in person with O blood group:**
    a. Anti-A antibody only [PGI May 2013]
    b. Anti-B antibody only
    c. Both Anti-A and Anti-B antibody
    d. No antibody
    e. Anti-O antibody

46. **Regarding IgE which of the following is false.**
    a. Cause anaphylaxis [AIIMS 2014]
    b. Immediate reaction
    c. Fix complement
    d. Cross placenta

Explanations and References with Illustrative Answers

1. **Ans. (a) IgG1** *Ref. Jawetz 27/e, p 137; Ananthanarayan 8/e, p 98, 9/e, p 97*

IgG

- Most abundant serum immunoglobulin (80%) with molecular weight of 1,50,000 and half-life of 21 days.
- Distributed equally between extravascular and intravascular compartment.
- Carbohydrate content is less if compared to other Ig.
- *IgG is divalent.*
- They are distributed as G1 (65%) > G2 (23%) > G3 (8%)> G4 (4%).
- The subclasses of IgG differ from one another in the size of hinge region and number and position of the interchain disulphide bond between the heavy chains.
- *IgG2* is directed against polysaccharide antigen, so it is important in defence against encapsulated bacteria.
- IgG is produced in secondary response of immunity.

Remember:
- Most abundant Ig in newborns–IgG.
- Only immunoglobulin which crosses placenta – IgG
- Basic structure of all Ig is 2 pairs of polypeptide chains (2H and 2L).

2. **Ans. (d) IgM** *Ref. Ananthanarayan 8/e, p 99, 9/e, p 98; Jawetz 27/e, p 137*

- Main immunoglobulin produced early in the primary response.
- **IgM**, composed of five H_2L_2 and 1 J chain and is heaviest immunoglobulin with molecular weight of 1000,000 (hence called millionaire molecule)
- It has valency of 10 (effective valency - 5, due to steritic hindrance)
- Its presence in the serum indicates recent infection (primary response).
- IgM is present on the surface of virtually all uncommitent B-cells.
- It is the most efficient immunoglobin for agglutinations, complement fixation.
- It has ***highest avidity*** among all Ig.
- *By 20th week, fetus produce IgM, IgD and receives maternal IgG so IgA and IgE are not present.*
- Phylogenetically it is the oldest immunoglobulin class.
- Treatment of serum with 0.12mM2 mercaptoethanol selectively destroys IgM. This is a simple method for differential estimation of IgG and IgM.

3. **Ans. (d) Covalent bond** *Ref. Ananthanarayan 8/e, p 102, 9/e, p 103*

Combination between* Ag *and* Ab *is firm but ***reversible,*** effected by the weaker intermolecular forces such as van der Waals forces, lonic bonds and hydrogen bonding, not by the firmer covalent bonds.

- The firmness of the union is influenced by affinity and avidity.
- *Affinity* is the intensity of attraction between antigen and antibody while *avidity* is the strength of bond after the formation of antigen-antibody complex.

4. **Ans. (d) Fetal infection is characterized by ↑in IgG** *Ref. Ananthanarayan 8/e, p 99, 9/e, p 96-98*

As IgM does'nt cross placenta, presence of IgM in fetus indicates fetal infection.

IgM	• Antigen receptor on B cells • Useful for diagnosing congenital infections • ***Earliest Ig*** to be synthesized by fetus • Increased in ***primary*** response • ***Max*** sedementation cofficient, max molecular weight and max intravascular distribution. • Exhibits strong classical complement fixation, opsonization, agglutination, lysis, immunohemolysis.

IgG	• Max serum conc. and half-life • Ig increase in ***secondary*** immune response and is opsonizing antibody. • Only Ig which ***crosses placenta*** so its presence in fetus indicates immunity not infection. • Exhibits strong precipitation and neutralization reaction.
IgE	• Ig increase in parasitic infection, allergic response • Only ***heat labile*** Ig • Max carbohydrate content
IgA	• Only Ig which is secreted by seromucinous gland such as saliva, tears, colostrum, respiratory and gastrointestinal secretions.

Remember:
- Ig present in milk: IgA and IgG
- Complement fixation: *Classical IgG* (except IgG4), IgM; *Alternate IgA*, IgD, IgG4.
- Polymeric form contains J chain: IgA, IgM.

5. **Ans. (b) Light chain** *Ref. Ananthanarayan 8/e, p 100, 9/e, p 99*

- BJP are identified in urine by its characteristic property of coagulation when heated at 50°C and dissolved at 70°C.
- BJP (abnormal Ig) are light chain of Ig (so may occur as kappa or lambda form) found typically in multiple myeloma.
- But in one patient, chain is either kappa or lambda, never both.

6. **Ans. (c) Kappa and Lambda chains** *Ref. Ananthanarayan 8/e, p 100, 9/e, p 99*

Already explained

7. **Ans. (b) and (d) Antibody excess and False-negative** *Ref. Ananthanarayan 8/e, p 104, 9/e, p 105*

Zone phenomenon (seen in agglutination and precipitation) consists of 3 parts:
i. **Prozone** = Ab excess = weak or absent precipitation reaction = False –ve
ii. **Zone of equivalence** = peak amount of precipitation.
iii. **Post zone** = Ag excess = weak or absent precipitation reaction.

8. **Ans. (c) 2 light chains and 2 heavy chains** *Ref. Ananthanarayan 8/e, p 96, 9/e, p 94*

Immunoglobulins are glycoprotein molecules consisting of four polypeptide chains:
- Two identical heavy chain (H) molecular weight > 50 KD_a
- Two identical light chain (L) molecular weight > 25 KD_a

H chains are structurally and antigenically distinct for each class

9. **Ans. (b) Hemagglutination inhibition test** *Ref. Ananthanarayan 8/e, p 111-16, 9/e, p 112, 117*

Following serological test, use labeled antibodies:

A. Immunofluorescence (IF) = Fluorescent Antibody Technique
- **Principle:** Labels (fluorescent dyes) conjugated with Antibodies of serum = labelled antibodies which is used to locate and identify *antigens* in tissues.
- Fluorescent dyes commonly used are ***fluorescein Isothiocynate and lissamine - rhodamine***

Immunofluorescence may be	
Direct IF test	**Indirect IF test**
• Antigen + labeled antibodies ↓ Antigen - Antibody complex is fluorescent e.g. detection of *rabies virus* antigen in brain smears	• Antigen + Antibody ↓ • Antigen - Antibody complex + fluorescent conjugated antiglobulin serum ↓ • Final product is fluorescent e.g. fluorescent *treponemal antibody* test for syphilis
• Disadvantage - Separate fluorescent conjugates have to be prepared against each antigen to be tested	• Advantage - A single antihuman globulin fluorescent conjugate can be used for detecting human antibody to any antigen

- Fluorescent dyes can also be conjugated with *complement*.
- Labelled complement can be used for detection of antigen or antibody.
- Antibody is detected by Sandwich technique.
- **Major disadvantage** of immunofluorescence is frequent occurence of nonspecific fluorescence in tissues and other materials.

B. **Radioimmunoassay (RIA) = Binder Ligand Assays**
- *Principle:* Radiolabelled ***Radioisotopes (MC) and enzyme*** conjugated to antigen / antibody = labelled reactants which ***measure*** antigen ***and*** antibodies.
- The substance (antigen) whose concentration is to be determined is termed the analyte or ligand.
- Binding protein (usually antibody) which binds to ligand is called the binder.
- RIA measure analytes up to picogram 10^{-12}g quantities.
- RIA has application in quantitation of hormones, drugs, tumor markers, IgE and viral antigens.
- Fixed amount of antibody reacts with radiolabelled antigen as well as varying known amount of unlabelled (test) antigen competitively.
- Concentration of test antigen is calculated from standard dose response or calibrating curve.

C. **Enzyme Immunoassays (EIA)**
- It is the most widely used procedure in clinical serology.
- It uses enzyme labelled antigen and antibodies as serological reagents, for the assay of antibodies and antigens.
- It is of two types:

i. **Homogenous EIA (one step test) -**
- Used only for assay of haptens such as drugs (opiates, cocaine, etc.)

ii. **Heterogenous EIA (multistep test) -**
- Major type of heterogenous EIA is **ELISA** which involves the use of immunosorbent specific for one of the components of reaction, the antigen or antibody.
- Immunosorbent may be particulate or solid phase

e.g.
- *Noncompetitive sandwich ELISA*
- ***Detection of rotavirus antigen in feces.***
- Anti-HIV antibody test.
- *Competitive ELISA*
- *IgM specific ELISA*
- *Capture ELISA*
- *Immunometric ELISA*
- *Card and dipstick methods*
- *Cylinder or* Casette *ELISA* for the detection of HIV type 1 and 2 antibodies. It is rapid.

D. **Chemiluminescence Immunoassay (CLIA)**
- Uses chemiluminescent compounds (such as luminol or acridinium esters) as the label to provide signal during antigen-antibody reaction.

E. **Immunoelectroblot Techniques**
- e.g. western blot test.

F. **Immunochromatographic Tests**
- Test system is a small cassette containing membrane impregnated with anti-HbsAg antibody - colloidal gold dye conjugate, ***e.g. HBsAg detection.***

G. **Immunoferritin Test**
- Antibody conjugate with ferritin.

H. **Immunoenzyme Test**
- Stable enzyme like peroxidase conjugated with antibodies.

Remember: *Hemagglutination inhibition test* - convenient method for detection and quantitation of antibody to the virus.

10. Ans. (b) H-chain *Ref. Ananthanarayan 8/e, p 96, 9/e, p 94*

It is the class of heavy chain (H) which determines class of Ig, e.g. gamma (γ), alpha (α), mu (μ), delta (δ), epsilon (ε) are H chain of IgG, IgA, IgM, IgD, IgE, respectively.

11. Ans. (d) IgA and IgG_2 deficiency *Ref. Harrison 18/e, p 2704, 19/e p 2108*

"IgG subclass deficiency may be suspected in children and adults who have a history of recurrent infections of the ears, sinuses, bronchi and/or lungs."

- Antibodies against the polysaccharide, coating (capsule) of certain disease-producing bacteria (e.g. the pneumococcus and Haemophilus influenzae) are predominantly of the IgG_2 type.
- These patients are unable to produce protective levels of antibody when immunized with unconjugated polysaccharide vaccines against Streptococcus pneumonaiae (the pneumococcus) or Haemophilus influenzae bacteria and are prone for infections by capsulated bacteria.
- Ig G_2 deficiency is frequently associated with deficiency of IgA.

Note: Overall IgA deficiency is the most common primary immunodeficiency.

12. Ans. (c) IgM *Ref. Harrison 17/e, p 2036; Ananthanarayan 9/e, p 98*

IgM in comparison to IgG :

- 500-1000 times more effective in opsonization
- 100 times more effective in bactericidal action
- 20 times more effective in bactericidal agglutination

Note: A single molecule of IgM can bring immune hemolysis whereas 1000 molecule of IgG molecules are required for some effect

13. Ans. (a) Good memory response *Ref. Understand the immune system Elgert (wiley/Black well)*

"Polysaccharide antigens are ineffective in producing immunogenic memory and this is the reason behind the protein conjugation of polysaccharide vacines."

Carbohydrate Polysaccharide antigens:

- T-cell independent antigens, hence they do not evoke T-cell response which is essential for memory response.
- Usually present in bacterial cell wall and capsule
- Stimulates B-cell directly without involving antigen presenting cells, or else produce polyclonal activation of B-cells.
- Considerably less immunogenic in comparison to peptide antigen
- Don't exhibit delayed type hypersensitivity
- In large doses induce tolerance

14. Ans. (d) Ag-Ab reaction can't occur in absence of electrolytes *Ref. Ananthanarayan 8/e, p 103, 9/e, p 104*

General features of Ag-Ab reaction:

- Reaction is specific, though specificity is not absolute.
- Entire molecules do not fragment but combine in the presence of electrolytes at specific pH and temperature.
- Combination occurs at the ***surface.***
- It is firm but ***reversible,*** firmness depends on affinity (intensity of attraction) and avidity (strength of bond)
- *Ab* are generally bivalent while Ag may have valency up to hundred.

Remember these tests:

Serological test	Type	Used to diagnose
i. AscolisThermoprecipitin test	Ring precipitation	Anthrax
ii. Lancefield test	Ring precipitation	Grouping of streptococci
iii. Kahn flocculation test	Tube precipitation	Syphilis
iv. VDRL test	Slide precipitation	Syphilis
v. Paul Bunnel test	Tube agglutination	Infectious mononucleosis
vi. Rose waller test	Passive hemagglutination	Rheumatoid arthritis
vii. Widal test	Tube agglutination	Typhoid
viii. Weil-Felix test	Heterophile tube agglutintion	Rickettsiae
ix. Wasserman reaction	Complement fixation test	Syphilis

15. Ans. (c) Immunological memory *Ref. See below*

- During the development of a primary immune respone to a pathogen, **memory cells are produced**.
- These lie dormant in the lymphatic system for many years.
- If they detect the same pathogen later on, they can clone rapidly and secrete antibodies.
- So, secondary exposure to a pathogen produces a much enhanced and rapid secondary response which kills the bacteria before clinical symptom appear.

- Vaccine takes advantage of this secondary response effect.
- Vaccine contains antigen from pathogens, which induces the production of memory cells–giving protection from the same pathogen, if encountered later on in life.

16. Ans. (b) Precipitation *Ref. Ananthanarayan 8/e, p 109-110, 9/e, p 105-109*

Serological reactions type		
	Precipitation	Agglutination
Pre-requisite	• Soluble antigen, antibody, electrolytes, at suitable temperature and pH	• Particulate antigen, antibody, electrolytes at suitable temperature and pH
Optimal proportion	• Precipitation is abundant and rapid if antigen and antibody are present in optimal or equivalent proportion	• Occurs optimally when antigens and antibodies react in equivalent proportion
Application	• Sensitive test for detection of antigen (can detect as tittle as 1 µg protein). Therefore used in forensic applications for identification of blood and seminal stains, testing for food adultrants	• Slide agglutination for blood grouping and cross matching. Tube agglutination is standard method for quantitative assessment of antibodies
Limitation	• Relatively less sensitive for detection of antibody	• More sensitive than precipitation for detection of antibodies

- Hemagglutination is a type of agglutination
- *Agglutination* (e.g. indirect HA) is *more sensitive* than precipitation (e.g. gel diffusion test) *for antibody* detection.

Remember:

Flocculation: – When instead of sedimenting, precipitate remains suspended as floccules, the reaction is known as flocculation.

Zone phenomen: – In agglutination reactions when either an antibody or antigen is in excess, agglutination does'ntoccur.

Passive agglutination: – The only difference between requirement for precipitation and agglutination reaction is the physical nature of antigen. By attaching soluble antigen to the surface of carrier particle, it is possible to convert precipitation test into agglutination test which are more sensitive for detection of antibodies. Such tests are known as passive agglutination tests.

17. Ans. (d) Increased in pneumococcal infection *Ref. Ananthanarayan 8/e, p 221, 9/e, p 81, 223*

- **CRP** (beta1 globulin, not antibody) is ***acute phase reactant*** (produced by hepatocytes). Its production is increased in bacterial infections, malignancies, tissue inflammation, rheumatic fever, etc. (i.e., non-specific inflammatory protein).
- C-reactive protein enhance phagocytosis by binding phospholipase, activates complement. In certain situation C-RP stimulates thrombosis and promotes of inflammatory cytokine production.
- CRP is detected by passive agglutinatoin using latex particles.

18. Ans. (c) Antigenic variation *Ref. Harrison 17/e, p 2035*

Learn it

19. Ans. (b) and (c) Tumor necrosis factor - alpha and Tumor necrosis factor - beta

"MHC class genes is classified as Class I, Class II, Class III."

Products of Class III genes includes:	• C_2 and C_4 of classical pathway • Heat shock protein • Soluble protein of complement system • Properdin factor B of alternative pathway • TNF alpha and beta.

20. Ans. (b) and (c) Fibrinogen and Haptoglobin

Ref. Ananthanarayan 8/e, p 86, 9/e, p 81; Infectious disease by Jonathan 2/e, p 856; Greenwood 18/e, p 114

Acute phase reactants (APR)

- APR is the generic name given to a approx. 30 biochemically different and functionally unrelated proteins which are synthesized and secreted by hepatocytes. Their level in the serum are either increased (positive APR) or reduced (negative APR), approx. 90 minutes after the onset of systemic inflammatory reaction. Some important APR includes:

i. C-reactive protein (B1 globulin)
ii. α 1 Antitrypsin
iii. Haptoglobin (α2 glycoprotein)
iv. Mannose binding protein
} ↑ **with inflammation** (Positive APR)
v. Serum anyloid
vi. α1 acid glycoprotein (orosomucoid)
vii. Fibrinogen
viii. Pre-albumin
ix. Albumin
x. Transferrin
} **Decreased with inflammation** (Negative APR)

These acute phase reactants enhance host resistance, prevent tissues injury, promotes repair of inflammatory lesions

21. Ans. (d) and (e) Hypervariable region and Idiotype region *Ref. Harper 24/e, p 746*

- Each H and L chain of Ig consists of variable (V) region/domain and constant (C) region/domain.
- H has 1 V_H and 3(CH1, CH2, CH3) constant region.
- L has 1 V_L and 1C_L region.
- *V_H and V_L* domain (formed by amino terminal portion) is specific antigen binding region (=Fab) (not antibody binding region as given in *Harrison, p 1922)*
- V_L and V_H region have hypervariable regions (hot-spots=extreme sequence variability) that constitute Ag binding 'Site' (not region) unique to each Ig molecule (at tip).
- L chain has 3 (in V_L) and H chain has 4 (in VH) Hypervariable regions. Also called as complementarity determinig regions (CDRs).
- Idiotype is specific region of Fab portion to which antigen binds.
- CH2 of IgG binds C1q in classical component, CH3 domain mediates adherence to monocyte surface.
- The area of H chain in C region between CH1 and CH2 is hinge region which cleaves by papain to form 1Fc and 2 Fab fragments.

22. Ans. (d) Plasma cells *Ref. Ananthanarayan 8/e, p 130, 9/e, p 92*

- **IgE** is antibody and all antibodies are produced by plasma cells which are oval, twice the size of small lymphocyte with eccentric nucleus containing large blocks of chromatin peripherally (cart wheel appearance). They are formed from B cells.
- *Don't Get Confused with Eosinophils* because eosinophils are activated by IgE, not produce it.

23. Ans. (a) and (c) IgG and IgM *Ref. Harrison 17/e, p 2036; Ananthanarayan 9/e, p 97*

- Classic complement pathway is not activated by IgG4 subtype.
- Alternate complement pathway is activated by IgA, IgD, IgG4.

24. Ans. (c) Formed by epithelial cell and plasma cell *Ref. Ananthanarayan 9/e, p 98*

IgA is of two types → **Serum IgA - Monomeric**, synthesized by plasma cells
→ **Secretory IgA** (SIgA) – 2 monomers joined by J chain

- Both J chain and dimeric SIgA are synthesized by plasma cells situated near the mucosal or glandular epithelium.

Note: There are two sub classes of IgA in human IgA_1 and IgA_2. In serum $IgA_1 > IgA_2$, whereas SIgA consist of equal amount of the two subclasses.

25. Ans. (b), (c) and (d) Half-life of IgG is 23 days, IgD is heat stable and IgE has highest carbohydrate content
Ref. Ananthanarayan 8/e, p 100, 9/e, p 96

"IgD has highest percent of carbohydrate but according to Harrison (17/e, p 2036) – it is IgE."

Half-life of Ig: IgG 23d > IgA 6d > IgM 5d > IgD > IgE.

26. Ans. (c) IgG *Ref. Ananthanarayan 8/e, p 100, 9/e, p 96*
Already explained

27. **Ans. (b) and (d) Beta - 1 globulin and Non specific inflammatory protein** *Ref. Ananthanarayan 7/e, p 218, 9/e, p 81*
Already explained

28. **Ans. (b) Widal Test** *Ref. Ananthanarayan 8/e, p 93*

The same or closely related antigen may sometimes occur in different biological species/classes, etc. It is called ***heterogenetic or heterophile antigen,*** e.g.

- Forssman antigen for *Pneumococcus, Salmonella.*
- Paul Bunnell test in infectious mononucleosis.
- *Streptococcus* MG agglutination.
- Weil Felix reaction (between proteus and *Rickettsia*).
- Cold agglutination test in primary atypical pneumonia.
- Antigen between *E. coli* and human RBC of B.

Remember: Widal test is tube agglutination test.

29. **Ans. (c) and (d) Antibodies appear in short time and persist for long** *Ref. Ananthanarayan 8/e, p 139, 9/e, p 146*
Secondary immune response is characterized by:

- Prompt, ***powerful,*** prolonged *response.*
- Much higher level of antibodies that last for *long periods.*
- ***Short,*** negligible *lag* phase.
- Ab is predominantly *IgG.*

30. **Ans. (b) Inhibits viral replication in cells** *Ref. Ananthanarayan 8/e, p 148, 9/e, p 155; Harrison 17/e, p 2025-26*
- **Interferon (host coded protein)** has no direct action on viruses but inhibit viral replication by selectively inhibiting translation of viral m-RNA without affecting cellular m-RNA.
- IFN are not virus specific but species specific.
- It is of 3 types:

Type	Cell source	Cell target	Biological activity
• *IFN α (protein) or leukocyte IFN*	All cells	All cells	Antiviral activity; stimulates T cell, macrophages and NK cell activity
• *IFN β (glycoprotein) or Fibroblast IFN*	All cells	All cells	Direct antitumor effects Upregulates MHC class I antigen expression. Used therepeutically in viral and autoimmune disease
• *IFNγ (glycoprotein) or immune IFN*	T cells NK cells	All cells	Regulates macrophage and NK cells activation Stimulates Ig secretion by B cells Induction of class II histocompatibility antigens TH1 T cell differentiation

31. **Ans. (a) Polysaccharide** *Ref. Ananthanarayan 8/e, p 92, 9/e, p 88*
- *Most* naturally Ag are ***proteins*** and ***polysaccharides.***
- Proteins are better Ag than polysaccharides.
- All proteins *except* ***gelatin*** is antigenic.
- Lipids and nucleic acids are also less antgenic, so their antigenicity is increased by combining with proteins.

32. **Ans (a), (b) (c) Specific, secondary response is rapid, variable region of Ig is involved in Ab production** *Ref. Ananthanarayan 8/e, p 152, 9/e, p 159; Greenwood 18/e, p 122*

Clonal selection theory of immune response
- During development B and T cells acquire specific cell surface receptor that commit them to a single antigen specificity. The lymphocytes are activated when they bind to their specific antigen.
- The lymphocyte reactives to any particular antigen, are only a small proportion of the total pool.
- When an antigen is encountered, the cell specific for that antigen gets activated, and trigger proliferation of cells with an identical genetic makeup (clones). This phenomenon is called as ***clonal selection.***
- *During embryonic life the lymphocyte clones acting against self-receptor are eliminated,* such clones are called ***forbidden clones.***
- For the mechanism of regulation of antibody response ***network hypothesis*** was postulated, according to which variable region of Ig carrying the antigen binding site is different in different antibodies. The distinct AA sequence at the Ag combining sites and the adjacent part of variable region are termed iditype. The idiotype in turn acts as antigenic determinent and can induce antidiotypic antibodies. These, in turn can induce antibodies against them and so on forming an idiotype network.
- Immunological memory is another consequence of clonal selection, due to which secondary response is rapid and heightened.
- Genetic basis of all this can be explained by ***split genes.***

33. Ans (c) Changes in heavy and light chain in constant region responsible for class and subtype *Ref. Ananthanarayan 8/e, p 101; DR Arora 3/e p 119*

Isotopic specificities

- The antigenic specificities which distinguish between the different classes and subclasses of immunoglobulin present in all individual of a species,. e.g. Antigen specificitv of IgA and IgG.
- They are located on (Constant) [illegible] in all individual.

[illegible]

[illegible] ortance is idiotypic specificity, pertaining to the nature of antigen binding site.

[illegible] which distinguishes immunoglobulins of the same class, between different groups of [illegible] ame species.

[illegible] ached to lateral aspect of TCR *Ref. Ananthanarayan 8/e, p 145, 9/e, p 90; Jawetz 27/e p 134*

[illegible] gens are certain antigens that can interact with antigen presenting cells and T-cells in non-specific manner. [illegible] ional antigens binds to the α β heterodimer groove of the MHC molecule through the V regions of TCR α and [illegible] ns, super antigens bind directly to the lateral aspect of the TCR β chain. Moreover, this activity does not involve [illegible] e endocytic processing required for typical antigen presentation.

- This interaction activates a larger number of T-cells (10%) than conventional antigen (about 1%) resulting in massive cytokine expression and immuno-modulation.
- Various superantigens include: Staphylococcal enterotoxin, toxic shock syndrome toxin, etc.

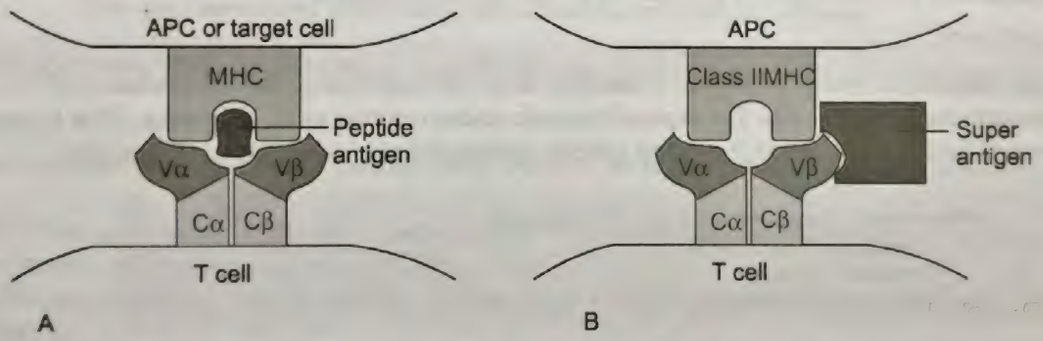


Fig. A. Interaction between peptide antigen. MHC, and ther T cell eceptor.
Fig. B. Interaction between a superantigen, MHC, and the T cell receptor

35. Ans. (c) Equivalence of antigen and antibody *Ref. Ananthanarayan 8/e, p 104, 9/e, p 104*

"Amount of precipitate formed is greately influenced by the relative proportions of antigen and antibodies, Precipitation reaction is rapid and abundant when antien and antibody are ***present*** in optimal or equivalent proportion."

Prozone : Zone of antibody excess

Peak : Zone of equivalence

Post zone: Zone of antigen excess

36. Ans (a; d) Western blot; ELISA *Ref. Ananthanarayan 8/e, p 103-113*

Precipitation reactions are seen in:

- Agglutination
- Precipitation
- Complement fixation
- ELISA
- Immunofluorescene (Director/indirect)
- Radioimmunoassay
- Chemiluminescence immunoassay (CLIA)
- Immunoblot techniques e.g. western blot

- Immunochromatographic test
- Immune electron microcopy tests

37. **Ans. (b) Distal part of alpha chain** *Ref. Ananthanarayan 8/e, p 133, 9/e p 139*

MHC I / HLA I

- Class 1 molecule consists of a three domains of heavy alpha peptide chain (a1; a2; a3) which are non-covalently linked to smaller β_2 microglobulin peptide.
- The ***distal domain*** (alpha 1 and alpha 2) of MHC 1 have highly variable amino acid sequences and are folded to form a cavity or groove. Protein antigens are bound to this groove for presentation to CD8 T cells.

MHC II / HLA II

- MHC II antigens are heterodimer consisting of an alpha and beta chain. Each chain has two domain proximal one is constant and distal one is variable.
- The two ***distal domain*** (alpha 1 , beta 1) *constitute the antigen binding site*, for recognition by CD4 T cells.
- Both class 1 and class 2 are members of immunoglobulin gene super family.

MHC III / HLA III

- MHC III are heterogenous they include complement components involved in the formation of C3 convertase, heat shock protein and tumor necrosis factor.

38. **Ans. (a) Capsular polysaccharide of S. pneumoniae** *Ref. Ananthanarayan 8/e, p 221, 9/e p 225*

'C' Reactive protein

An abnormal protein (beta 1 globulin) that precipitates with the somatic 'C' antigen of pneumococci, appears in the acute phase of pneumonia and disappears during convalescence. This protein found in other conditions also is called as C-reactive protein, where C stands for the C substance of pneumococci. This C-RP is not an antibody, rather a acute phase reactant synthesised in liver in response to bacterial infection, inflammation.

What is C substance?

Cell wall of pneumococci contains two types of teichoic acid; one exposed on the cell surface and the other similar form covolently bound to membrane lipids. The exposed teichoic acid is referred as 'C' substance. This 'C' substance is common to all pneumococci and is unrelated to the group specific carbohydrate of β hemolytic streptococci.

Remember: Pneumococci has three antigenic determinants:
- Capsular polysaccharide
- Somatic M protein
- Cell wall carbohydrate

So it is clear that 'C' does not stands for any of the choice mentioned in question, as only option 'a' relates it to pneumococci it can be taken as correct answer.

39. **Ans. (b) IgM** *Ref. Jawetz 25/e, p 130, 27/e p 98*

IgM is the first antibody to be produced, after exposure to both antigen or allergin

"IgM is the first antibody formed in every response" *...Medical Microbiology by BS Napoba*

Thogh ovaalbumin is an allergin, its initial injection would produce IgM first, then IgE. If it is injected again IgE production would be first response.

40. **Ans. (a, c, d and e) Stimulation of Toll-like receptors, Increase both adaptive and innate immune response, Activate both B and T lymphocyte, Ensure prolonged delivery of antigen** *[Ref: Ananthanarayan 8/e143-44: Medical microbiology by Greenwood 16/e110, 671*

Role of Adjuvants

- Adjuvants are substances that stimulate the immune response, They enhance the immunogenicity of a vaccines for example, by facilitating uptake into antigen-presenting cells.
- Adjuvants are essential for enhancing and directing the adaptavie immune response to vaccine antigens, which is mediated by both B and T cells
- Due to the variety of mechanism and links between the innate and adaptive immune response, an adjuvant-enhanced innate immune response results in an enhanced adaptive immune response.

- **Adjuvants exert their immune-enhancing effects by following immune-functional activities:**
  1. Adjuvants help in the translocation of antigens to the lymph nodes where they can be recognized by T cells. Thereby producing greater T cell activity
  2. Adjuvants provide physical protection to antigens which grants the antigen a prolonged delivery. This means that the cell will be exposed to the antigen for a longer duration.
  3. They increase the capacity to cause local reactions at the injection site (during vaccination), inducing greater release of danger signals like chemokines.
  4. They induce the release of inflammatory cytokines which helps to not only recruit B and T cells at sites of infection but also to increase transcriptional events leading to a net increase of immune cells as a whole.
  5. Finally they are believed to increase the innate immune response to antigen by interacting with pattern recognition receptors (PRRs), specifically Toll-like receptors (TLRs), on accessory cells.

41. Ans. (c) Differential RNA processing *Ref. Internet*

Membrane bound versus secreted immunoglobulin

- A primitive B-cell (virgin B cell) bears IgM (and possibly IgD) in its membrane; however after stimulation, it begins to secrete IgM into its surrounding environment.
  These two form of IgM are different:
- The secreted form of IgM has a different C-terminal sequence which, lacks a membrane anchoring region.
- Membrane bound IgM is not capable of associating with J Chain and forming its normal pentameric structure. So, membrane bound IgM remains exclusively in monomeric form.
  These two forms differs basically in their ***mu***-chain which is synthesized to via an alternative splicing scheme.

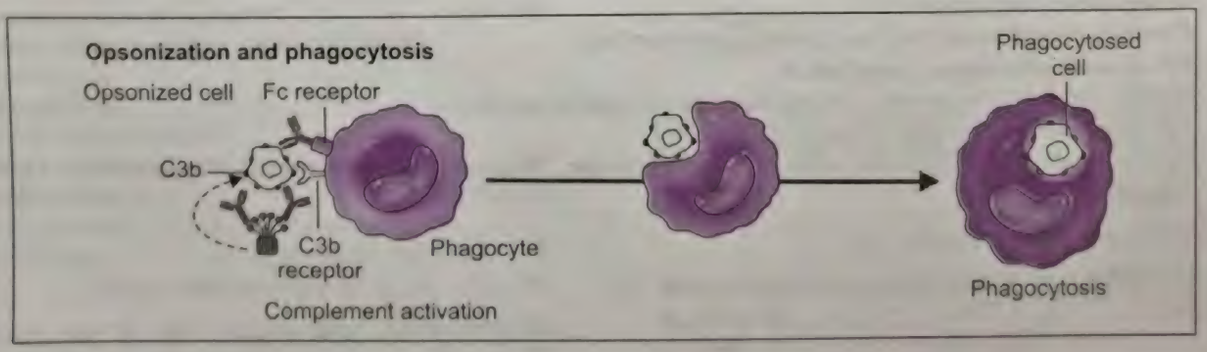
Other options

- *Allelic Exclusion:* Though diploid cells have two copies of every immunoglobulin gene, only one of the two is expressed in a given B cell. This allelic exclusion ensures the symmetry of antibody.
- *Class switching:* A particular antibody forming cell can switch from production of IgM to IgG from IgD to IgA, etc. In this switch only CH (heavy chain) changes, so that the original combining site remains same, however gets associated with a molecule of a different class or subclass.

Note: Such switching is undirectional, i.e. once IgM cell begin to secrete IgG, it can't go back to secrete IgM.

42. Ans. (a) 3b and Fc *Ref. Robbin's 8/e 202*

- Cells coated by antibodies are cleared through phagocytosis. These bacterial cells are recognized by phagocytic Fc receptors.
- In addition when IgM on IgG antibodies are deposited on the surfaces of cells they may activate the complement system by the classical pathway and generates C3b and C4b.
- C3b and C4b gets deposited over the surface of bacterial cell and are recognized by the phagocytes. The net result is phagocytosis.



Remember: Antibody mediated destruction of cells may occur by antibody dependent cellular cytotoxicity (ADCC) also, where cells are lysed with out phagocytosis. ADCC is mediated by monocytes, neutrophills, eosinophils and NK cells.

43. Ans. a and d, i.e. Widal test, ELISA *Ref. Ananthanarayan 9/e 109*

Heterophile Antigen: Similar antigen present on dissimilar organisms are heterophile antigens.

Heterophile Antibodies: Antibodies reacting with heterophile antigens are called heterophile agglutinations.

Heterophile Agglutination: Antibodies derived from one species reacting with heterophile antigen of another species causing agglutination.

Example: Agglutination of horse RBC's by the heterophile antibodies present in infectious mononucleosis patient serum.

Heterophile Agglutination Tests		Application
Weil-felix reaction	– Typhus rickettsiae, some strains of proteus bacilli	– Serodiagnosis of Typhus fever
Paul-Bunnel test	– Sheep horse red cell agglutinins in the serum of infectious mononucleosis patient	– Infectious mononucleosis
Cold Agglutination test	– Test is positive in mycoplasma pneumoniae. The patients sera agglutinate human O group erythrocytes at 4°C, the agglutination is reversible at 37°C	– Mycoplasma
Streptococcus Mg agglutination test	– For diagnosis of primarily atypical pneumonia	

44. Ans. (a, d and e) IgM is produced..., IgG is main..., IgA protects... *Ref. Ananthanarayan 9/e 96*

Already explained

45. Ans. (c) Both Anti-A and Anti-B antibody *Ref. Ananthanarayan 9/e 191*

Distribution of ABO antigen and antibodies in red cells and serum				
Group	*Red cells*		*Serum*	
	Antigen present	*Agglutinated by serum of group*	*Antibody present*	*Agglutinates cells of group*
A	A	B, O	Anti-B	B, AB
B	B	A. O	Anti-A	A, AB
AB	A and B	A, B, O	None	None
O	None	None	Anti-A and Anti-B	A, B, AB

46. Ans. (d) Cross placents *Ref. Ananthanarayan 9/e 99*

IgE

- 85 molecule immunoglobulin with a half life of two days and molecular wright about 190,000
- Structurally resemble IgG
- Heat labile and affinity for the surface of tisue cells
- Suceptible to mercaptoethanol.
- Does not pass the placental barrier or fix complement.
- Mostly extravascular in distribution.
- Mainly produced by linings of respiratory and intestinal tracts.
- IgE is responsible for type I hypersensitivity.
- Protect against pathogens by mast cell degranulation. Believed to have a special role in defence against helminth infections.

Chapter Review

1. **The use of monoclonal antibodies is:** [PGI 90]
   a. Immunotherapy
   b. Immunological identification of cells and tissues
   c. Radioimmunoimaging
   d. All of the above *[Ref. Ananthanarayan 8/e, p 113, 9/e, p 112; Greenwood 18/e, p 125; Harrison 17/e, p 2043]*

2. **Most sensitive test for antigen detection is:** [PGI 90]
   a. RIA
   b. ELISA
   c. Immunofluorescence
   d. Passive hemaglutination
   [Ref. Ananthanarayan 8/e, p 113, 9/e, p 113]

   - RIA permits the measurement of analytes (antigen) up to picogram quantities.

3. **Reaction of soluble antigen with antibody is known as:** [AI 90]
   a. Agglutination b. Precipitation
   c. Flocculation d. Complement fixation
   [Ref. Ananthanarayan 8/e, p 104, 9/e, p 105]

4. **Secretory piece of IgA is synthesized in:** [PGI 93]
   a. T-cells b. B-cells
   c. Lymph nodes d. Mucosal epithelium
   [Ref. Ananthanarayan 8/e, p 99, 9/e, p 97]

5. **Life-long immunity is seen in all of the following except:** [PGI 93, Bihar 93]
   a. Pertusis b. Mumps
   c. Rubella d. Plague
   [Ref. Park 22/e, p 1271]

6. **Prozone phenomenon is due to:** [AI 93]
   a. Disproportionate antigen-antibody levels
   b. Excess antigen
   c. Excess antibody
   d. Hyperimmune reaction
   [Ref. Ananthanarayan 8/e, p 104, 9/e, p 105]

7. **M band is absent in:** [PGI 95]
   a. IgG myeloma b. IgM myeloma
   c. IgA myeloma d. Light chain disease
   [Ref. Ananthanarayan 8/e, p 100, 9/e, p 99]

8. **The exact part of the antigen that reacts with the immune system is called as:** [Karnat 01]
   a. Clone
   b. Epitope
   c. Idiotope
   d. Effector
   [Ref. Ananthanarayan 8/e, p 91, 9/e, p 188]

9. **Rapid serological diagnostic test includes all except:**
   a. Latex agglutination [UP 01]
   b. Spectrophotometry
   c. Gel electrophoresis
   d. Radioimmuno assay
   [Ref. UInder consideration]

10. **Immunoglobulin found in B lymphocyte:** [UP 01]
    a. IgA b. IgE
    c. IgG d. IgD
    [Ref. Ananthanarayan 8/e, p 100, 9/e, p 98]

11. **Variable protion of antibody molecule is:** [UP 01]
    a. C-terminal
    b. N-terminal
    c. Intermediate region
    d. Carbohydrate moiety
    [Ref. Ananthanarayan 8/e, p 97, 9/e, p 94]

12. **Portion of immunoglobulin molecule with molecular weight of 50,000:** [JIPMER 01]
    a. Secretory piece
    b. H chain
    c. L chain
    d. J piece
    [Ref. Ananthanarayan 8/e, p 96, 9/e, p 94]

13. **Function of IgA is:** [Kerla 01]
    a. Acts as a mucosa barrier for infection
    b. Circulating antibody
    c. Kills virus infected cells
    d. Activates macrophages
    e. Causes delayed hyper sensitivity reaction
    [Ref. Ananthanarayan 8/e, p 98, 9/e, p 98]

14. **The following constitutes approximately 75% of total immunoglobin in humans:** [Karnat 02]
    a. IgG b. IgM
    c. IgE d. IgA
    [Ref. Ananthanarayan 8/e, p 97 9/e, p 186]

15. **Immunoglobin are involved in type 1 hypersensitivity reaction is:** [DNB 2013]
    a. IgG b. IgM
    c. IgA d. IgE
    [Ref. AA, 9/e, p 163]

Answers 1. d. All ... 2. a. RIA 3. b. Precipitation 4. d. Mucosal 5. d. Plague
6. c. Excess ... 7. d. Light ... 8. b. Epitope 9. b. Spectro ... 10. d. IgD
11. b. N-terminal 12. b. H chain 13. a. Acts ... 14. a. IgG 15. d. IgE

16. Structure of antibodies is composed of: [UP 03]
a. Single peptide chain
b. Two peptide chains
c. Non sulphur amino acid
d. 2 long and 2 short peptide chain
[Ref. Ananthanarayan 8/e, p 96, 9/e, p 94]

17. Isoantigens are: [UP 05]
a. Found in some but all members of species
b. Found in some but not all members of species
c. Occurs in different biological species, class and kingdoms
d. All individuals in a particular species
[Ref. Ananthanarayan 8/e, p 93, 9/e, p 69]

18. Paul Bunnell antibodies are reactive in all except: [SGPGI 05]
a. OX
b. Sheep
c. Dog
d. Horse
[Ref. Ananthanarayan 8/e, p 477, 9/e, p 476]

19. Antibodies are most responsive to: [SGPGI 05]
a. Recipients tissue
b. Autograft
c. Isografts
d. Allograft
[Ref. Ananthanarayan 8/e, p 178, 9/e, p 184]

20. First immunoglobulin to appear following infections: [SGPGI 05]
a. IgG
b. IgM
c. IgA
d. IgE
[Ref. Ananthanarayan 9/e, p 98]

21. The exact part of the antigen that reacts with immune system is called as: [Kar 01]
a. Clone
b. Epitope
c. Idiotype
d. Effector
[Ref. Ananthanarayan 8/e, p 91, 9/e, p 88]

22. Which of the following is a flocculation test: [Kar 01]
a. Widal test
b. Weil-Felix test
c. VDRL
d. Paul-Bunnell test
[Ref. Ananthanarayan 8/e, p 105, 9/e, p 105]

23. The following constitutes approximately 75% of total immunogobulin in humans: [Kar 02]
a. IgG
b. IgM
c. IgE
d. IgA
[Ref. Ananthanarayan 8/e, p 97, 9/e, p 96]

24. Which immunoglobulin is least important in human beings: [Kolkata 03]
a. IgE
b. IgD
c. IgG
d. IgA
[Ref. Ananthanarayan 9/e, p 98, 8/e, p 100]

25. Opsonization occurs due to: [Bihar 04]
a. Endotoxin
b. Complement
c. IgM
d. IgG
[Ref. Aanthnarayan 9/e, p 112, 8/e, p 100]

26. Immunoglobulin present in local secretions is: [DNB 2010]
a. IgG
b. IgA
c. IgM
d. IgD
[Ref. AA 8/e 98, 9/e, p 97]

27. In-utero infection leads to which immunoglobulin: [DNB 2012]
a. IgM
b. IgA
c. IgG
d. IgD
[Ref. AA 8/e 99, 9/e, p 98]

28. Atopy is mediated by: [Jharkhand 06]
a. IgE
b. IgD
c. IgM
d. IgA

29. Bronchial secretion secretes: [UP 2007]
a. IgA
b. IgE
c. IgM
d. IgG
[Ref. Ananthanarayan 8/e, p 90, 9/e, p 97]

30. Neutralization test is: [Delhi 2008, 2007]
a. VDRL
b. ASLO
c. Rose Waller test
d. Widal test
[Ref. Ananthanarayan 8/e, p 111, 9/e, p 112]

31. VDRL test is a: [RJ 2005]
a. Flocculation
b. Agglutination
c. Complement fixation
d. None

32. IgM appears in fetus at what gestational age:
a. 10 weeks
b. 20 weeks
c. 30 weeks
d. at birth
[Ref. Ananthanarayan 9/e, p 98]

Answers 16. d. 2 long ... 17. b. Found ... 18. a. OX 19. d. Allograft 20. b. IgM
21. b. Epitope 22. c. VDRL 23. a. IgG 24. b. IgD 25. b. Complement
26. b. IgA 27. a. IGM 28. a. IgE 29. a. IgA 30. b. ASLO
31. a. Flocculation 32. b. 20 weeks

NEET Pattern Questions

1. **Most abundant immunoglobulin in human body:**
   a. IgM b. IgG1
   c. IgG2 d. IgG3
   [Ref. Ananthanarayan, 9/e, p 96-97]

IgG contribute 80% of the total serum immunoglobulins. There are four subclasses IgG1, IgG2, IgG3, IgG4 which are disbributed in human serum in the approximate proportions of 65%, 23%, 8% and 4% respectively.

2. **When a particulate antigen combines with an antibody in approximate proportion, the resulting reaction is:**
   a. Agglutination b. Prozone phenomenon
   c. Precipitation d. Floculation
   [Ref. Ananthanarayan, 9/e, p 108]

3. **Immunoglobulin changes in variable region:**
   a. Idiotype b. Isotope
   c. Allotype d. Epitope
   [Ref. Harrison, 18/e, p 2673]

Variability of amino acid sequence in the variable region form the sepcific antigen binding site idiotype

4. **Maximum half life:**
   a. IgG b. IgA
   c. IgM d. IgE
   [Ref. Ananthanarayan, 9/e, p96]

5. **Pentameric structure green wood:**
   a. IgM b. IgG
   c. IgA d. IgD
   [Ref. Greenwood, 18/e, p 102, Ananthanarayan, 9/e, p98]

IgM are polymers of five four peptide subunits, each bearing on C_{μ} domain

6. **Which of the following immunoglobulin is responsible for opsonisation:**
   a. IgA b. IgG
   c. IgM d. IgE
   [Ref. Ananthanarayan, 9/e, p 98]

IgM is 500–100 times more effective in opsonization.

7. **Immunoglobulin isotype class switching is determined by:**
   a. Constant region of light chain
   b. Constant region of heavy chain
   c. Variable region of light chain
   d. Variable region of heavy chain
   [Ref. Ananthanarayan, 9/e, p 101]

8. **Antigen idiotype is related to:**
   a. Fc fragment
   b. Hinge region
   c. C-terminal
   d. N-terminal
   [Ref. Ananthanarayan, 9/e, p 95, Fig 11.3]

9. **A child presents with recurrent episodes of sinopulmonary infection by bacteria with polysacchride rich capsule. Deficiency of which of the following immunoglobulin subclasses should be specifically investigated:**
   a. IgA b. IgG1
   c. IgG2 d. IgA + IgG2
   [Ref. Harrison, 18/e, p 2704]

10. **Nagler reaction is type of:**
    a. Neutralization reaction
    b. CFT
    c. Precipitation
    d. Agglutination *[Ref. Ananthanarayan, 9/e, p 112]*

11. **Coombs test is:**
    a. Precipitation test
    b. Agglutination test
    c. CFT
    d. Neutrilization test *[Ref. Ananthanarayan, 9/e, p 109]*

12. **Heterophile antibody is found in:**
    a. Well-Felix test
    b. Widal test
    c. VDRL
    d. All *[Ref. Ananthanarayan, 9/e, p 108]*

13. **Monoclonal antibody binds to:**
    a. Epitope
    b. Paratope
    c. Both epitope and paratope
    d. None of the above *[Ref. Ananthanarayan, 9/e, p 87]*

14. **Which of the following is agglutination test:**
    a. Widal test b. VDRL
    c. Ascoli's test d. Kahn test
    [Ref. Ananthanarayan, 9/e, p 109]

15. **Prozone phenomenon is seen with:**
    a. Same concentration of antibody and antigen
    b. In antigen excess to antibody
    c. Antibody excess to antigen
    d. Hyperimmune reaction
    [Ref. Ananthanarayan, 9/e, p 103]

Answers 1. b. IgG1 2. a. Agglutination 3. a. Idiotype 4. a. IgG 5. a. IgM
6. c. IgM 7. b. Constant region 8. d. N-terminal 9. d. IgA + IgG2 10. a. Neutralization
11. b. Agglutination test 12. a. Well-Felix test 13. a. Epitope 14. a. Widal test 15. c. Antibody excess...

16. Quantitative estimation of antibody is done by:
a. Ziehl-Neelsen procedure
b. Ouchterlony procedure
c. Halden procedure
d. Spaulding procedure

Ouchterlony procedure:
- Diffusion of both antigen and antibody in two dimensions.
- It is used to detect identity, cross reaction, and non-identity between differrent antigen in a reacting mixture.
- It is one of the simplest technique to determine the presence and litre of any antibody in serum.

17. Gammaglobulins are synthesized in:
a. Liver b. Lung
c. Plasma cells d. Spleen
[Ref. Jawetz, 27/e, p 138]

18. Pentameric structure:
a. IgM
b. IgG
c. IgA
d. IgD *[Ref. Jawetz, 27/e, p 137]*

19. IgE binds to which cell:
a. T cells
b. B cells
c. Mast cells
d. NK cells

[Ref. Jawetz, 27/e, p 137]

Fc region of IgE binds to the high affinity recepter or the surface of mast cells.

20. Neutrilization test us:
a. Widal test b. Weil-Felix test
c. Paul Bunnel test d. Nagler reaction
[Ref. Ananthanarayan, 9/e, p 112]

Nagler reaction is a neutratization test for identification of alpha toxin of Cl. perfringens

Note: Schick test is another neutralization test.

21. Which of the following is a superantigen:
a. Exfoliative toxin of Staphylococcus
b. Lipopolysaccharide of gram negative bacteria
c. Enterotoxin of V cholerae
d. Shiga toxin of EHEC *[Ref. Ananthanarayan, 9/e, p 91]*

22. Rose-Waaler test is:
a. Complement fixation test
b. Pricipitation in gel
c. Ring precipitation *[Ref. Ananthanarayan, 9/e, p 110]*
d. Passive hemagglutination test

Rose-Waaler test is passive hemogglutination test for detection of RA factor

23. Example of agglutination test:
a. Widal test b. Schick test
c. VDRL test d. Ascoli's test
[Ref. Ananthanarayan, 9/e, p 109]

24. Example of precipitation test is:
a. Rose-Waaler test
b. Widal test
c. Latex agglutination
d. Kahn test *[Ref. Ananthanarayan, 9/e, p 106]*

25. Immunoglobulin variation does not depend on:
a. Light chain
b. Heavy chain
c. Amino acid sequence
d. Constant region *[Ref. Ananthanarayan, 9/e, p 96]*

26. Molecular mass of IgG (in K Da):
a. 150 b. 400
c. 1000 d. 1500

27. Weil-Felix reaction is:
a. Ring test
b. Tube precipitation test
c. Slide agglutination test
d. Tube agglutination test

Can be done as either a slide or tube method

28. Lattice phenomenon is seen in:
a. Neutrilization reaction
b. Complement fixation test
c. Precipitation test
d. All of the above *[Ref. Ananthanarayan, 9/e, p 105]*

29. Which is an example of antigen-antibody reaction:
a. Flocculation reaction
b. Precipitation
c. Agglutination
d. All of the above *[Ref. Ananthanarayan, 9/e, p 104]*

30. Prozone phenomenon is a feature in:
a. Tularemia
b. Legionnaire's disease
c. Plague
d. Secondary syphilis *[Ref. Ananthanarayan, 9/e, p 374]*

31. Antigen-antibody binding occurs at:
a. Surface b. Center
c. Inside molecule d. Anywhere in structure
[Ref. Ananthanarayan, 9/e, p 104]

32. Which of the following is a complement fixation test:
a. Wassermann test for syphilis
b. VDRL test for syphilis
c. Kahn test for syphilis
d. Rose-Waaler test for RA
[Ref. Ananthanarayan, 9/e, p 110]

Answers 16. b. Ouchterlony... 17. c. Plasma cells 18. a. IgM 19. c. Mast cells 20. d. Naglar reaction
21. a. Exfoliative toxin... 22. d. Passive hemag... 23. a. Widal test 24. d. Kahn test 25. d. Constant region
26. a. 150 27. c and d. ... 28. c. Precipitation... 29. d. All of the above 30. d. Secondary syphilis
31. a. Surface 32. a. Wassermann ...

33. Prozone phenomenon is responsible for
a. False negative test
b. False positive test
c. May cause any of the above
d. Has no relation with accuracy of test

34. Examaple of neutrilization reaction:
a. VDRL
b. Widal test
c. Kahn test
d. Nagler reaction
[Ref. Ananthanarayan 8/e, p 112]

35. Oakley-Fulthorpe procedure is:
a. Agglutination test
b. Precipitation test
c. Single diffusion in one dimension
d. Double diffusion in one dimension

36. Haptens are immunogenic when they covalently bind to:
a. Lipid carrier
b. Polysaccharide carrier
c. Protein carrier *[Ref. Ananthanarayan 9/e, p 87]*
d. Any of the above carrier

Answers **33.** a. False negative test **34.** d. Nagler reaction **35.** d. Double diffusion in one dimension
36. d. Any of the above carrier

CHAPTER

38

Hypersensitivity

Hypersensitivity is defined as immunologic tissue injury in response of subsequent exposure with the allergen (shocking or challenge dose). It is of following types:

Types	Mechanism and Effects	Examples
1. Type I (Immediate or reaginic HSN) Systemic acute anaphylaxis Local anaphylaxis = atopy	• **Ab -** IgE (cytotropic) • **Cells -** IgE B cell, mast cells, basophils, eosinophil • **Pivotal role -** by T_H2 cell • Most important **vasoactive amine:** Histamine • Slow reacting substance of anaphylaxis ***(SRS-A)*** = Leukotrienes ***($LT B_4, C_4, D_4, E_4$)***	• Urticaria; angioedema; hay fever and some forms of asthma; eczema • Anaphylactic shock • Casoni's test, • Theobald Smith phenomenon • Schultz Dale phenomenon • Prausnitz Kustner (PK) reaction
2. Type II [cytotoxic or cytolytic] HSN a. Complement dependent	• **Ab:** IgG or IgM • Lysis or phagocytosis by opsonization • Most commonly involves blood cells (**Mnemonic:** II HSN involves blood: b is second alphabet)	• Transfusion reactions • Erythroblastosis fetalis (Hemolytic disease of neonates) • AI hemolytic anemia or agranulocytosis or thrombocytopenia; • Pemphigus syndrome • Bullous pemphigoid • Pernicious anemia • Thrombotic phenomenon • Acute rheumatic fever • Some forms of vasculitides and certain drug reactions.
b. Type VI HSN = Antibody dependent cell mediated cytotoxicity (ADCC)	• **Ab -** usually IgG sometimes IgE • Cell lysis without phagocytosis by monocytes, neutrophil, eosinophils and NK cells	• Phagocytosis of tumor cells or parasite • Some role in graft rejection
c. Type V HSN = Antibody mediated cellular dysfunction.	**Antireceptor Antibody** • Stimulation • Inhibition	• Thyrotoxicosis/Graves' diseases • Myasthenia gravis
3. Type III [Immune complex] HSN Local immune complex disease [= Arthus reaction] Systemic immune complex [Serum sickness]	• **Ab** - IgG or IgM • Ag-Ab complex activate complement → attack neutrophil → release of lysosomal enzymes	• SLE • Certain forms of acute glomerulonephritis • Rheumatoid arthritis • Hyperacute graft rejection • Hypersensitivity pneumonitis • Infective endocarditis • PAN • Henoch-Schonlein purpura • Schick test • Type 2 lepra reaction (ENL)
4. Type IV [Cell mediated] HSN a. Delayed type by CD4 T_H1 - Induration is its characteristic	• Ab - No antibody • Initiated by specifically sensitized T lymphocytes	• Tuberculin test • Lepromin test • Fairleys (Schistosomiasis) test • Frie's (LGV) test • Granulomatous inflammation • Contact dermatitis • Defense against intracellular pathogen • Type I lepra reaction
b Cell mediated cytotoxicity by CD - 8 T cells		• Graft rejection • Resistance to virus infection • Tumor immunity

* ***HSN:*** Hypersensitivity

Multiple Choice Questions

1. **Examples of type I hypersensitivity is:** [AI 98]
   a. Lepromin test b. Tuberculin
   c. Casoni's test d. Arthus reaction

2. **Which of the following is an example of type IV hypersensitivity?** [AI 97]
   a. Arthus reaction
   b. Serum sickness
   c. Schwartzman reaction
   d. Granulomatous reaction

3. **Which of the following is an example of type IV hypersensitivity?** [AI 96]
   a. Granulomatous reaction
   b. Schwartzman reaction
   c. Arthus reaction
   d. Serum sickness

4. **Which of the following is false?** [AI 95]
   a. Theobald-Smith phenomenon is a type I hyper-sensitivity reaction
   b. Serum sickness is a type II hypersensitivity reaction
   c. Allograft rejection is a type IV hypersensitivity reaction
   d. Transfusion reaction is a type II hypersensitivity reaction

5. **Hemolytic disease of newborn is which type of hypersensitivity reaction?** [PGI June 07]
   a. Type - I
   b. Type - II
   c. Type - III
   d. Type - IV
   e. Type - V

6. **Skin test is used for which hypersensitivity reaction?** [PGI June 07]
   a. I b. II
   c. III d. IV

7. **Skin test based on neutralization reaction is/are:**
   a. Casoni test b. Lepromin test [PGI 04]
   c. Tuberculin test d. Schick test

8. **Type-1 hypersensitivity includes all of the following except:** [PGI 00]
   a. Autoimmune hemolytic anemia
   b. Anaphylaxis
   c. Extrinsic asthma
   d. Hay fever

9. **True about anaphylaxis:** [PGI 00]
   a. Type-1 reaction
   b. Large amount of histamine released
   c. Cytokines like IL4, IL5, and IL6 and GMCSF are released
   d. Mediated through allergen specific IgE

10. **Disorders of phagocytosis are all, except:**
    a. Job's syndrome [PGI May 2013]
    b. Chediak-Higashi syndrome
    c. Myeloperoxidase deficiency
    d. Wiskott-Aldrich syndrome
    e. Tuftsin deficiency

11. **Anaphylaxis is mediated by:**
    a. 5-hydroxytryptamine [PGI May 2013]
    b. Heparin
    c. Prostaglandin
    d. Platelet activating factor

Explanations and References with Illustrative Answers

1. **Ans. (c) Casoni's test** *Ref. Taylor 3/e, p 107*

Type I HSN (IgE mediated)	Type II HSN (IgG and IgM mediated)
• Eczema • Hay fever • **Asthma** (atopy) • Urticaria • Anaphylactic shock • Acute dermatitis • ***Theobald Smith phenomenon*** • PK **(Prausnitz Kustner)** reaction • Casoni's skin test • **Schultz** Dale phenomenon	• **Blood transfusion reactions** • Erythroblastosis fetalis • **AI Hemolytic anemia** or agranulocytosis or thrombocytopenia • Pemphigus vulgaris • **Good pasture** syndrome • Bullous pemphigoid • Pernicious anemia • **Acute rheumatic** fever • Diabetes mellitus • **Graves disease** • Myasthenia gravis
Type III HSN (IgM or IgG mediated)	**Type IV HSN (cell mediated)**
• Local - **Arthus** reaction • **Systemic-serum sickness** • Lepromin skin test • Schick skin test • PAN • **Rheumatoid** arthritis • SLE • Acute viral hepatitis • Penicillamine toxicity • **Hyperacute graft rejection** • Type 2 lepra reaction (ENL) • Hypersensitivity pneumonitis	• Tuberculin skin test • Lepromin skin test • Contact dermatitis • Jones Mote reaction **(cutaneous basophilic HSN)** • **TB** • Sarcoidosis • Temporal arteritis • Patch test • **Granulomatous inflammation** • Type I lepra reaction

2. **Ans. (d) Granulomatous reaction** *Ref. Ananthanarayan 8/e, p 168, 9/e, p 168*
Schwartzman reaction is **not an immune reaction** but alteration in factors (**e.g.** massive activation of complement) affecting intravascular coagulation, e.g. Purpuric rashes of meningococcal septicemia; Waterhouse Friderichsen syndrome.
Ref. Answer no. 1 for other options

3. **Ans. (a) Granulomatous reaction** *Ref. Ananthanarayan 9/e, p 167*
Already explained

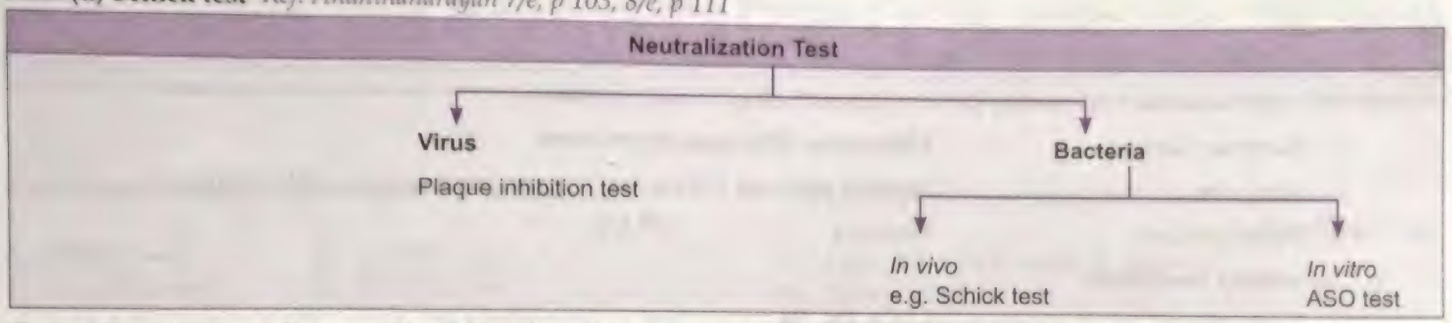
4. **Ans. (b) Serum sickness is type II HSN** *Ref. Taylor 3/e, p 119*

Type of Rejection	Type of HSN	Target Sites in Transplantation
• Hyperactue rejection (preformed Ab against donor transplantation Ag)	Type II cytotoxic Type III HSN	Small blood vessels in donor tissues
• Acute rejection	Type II cytotoxic Type III HSN	Parenchymal cells Small blood vessels
• Chronic rejection	Type III HSN Type IV HSN	Small blood vessels

5. **Ans. (b) Type - II** *Ref. Taylor 3/e, p 107*
Already explained

6. **Ans. (a), (c) and (d) I, III and IV** *Ref. Taylor 3/e, p 107; Jawetz 24/e, p 142-43*
Already explained

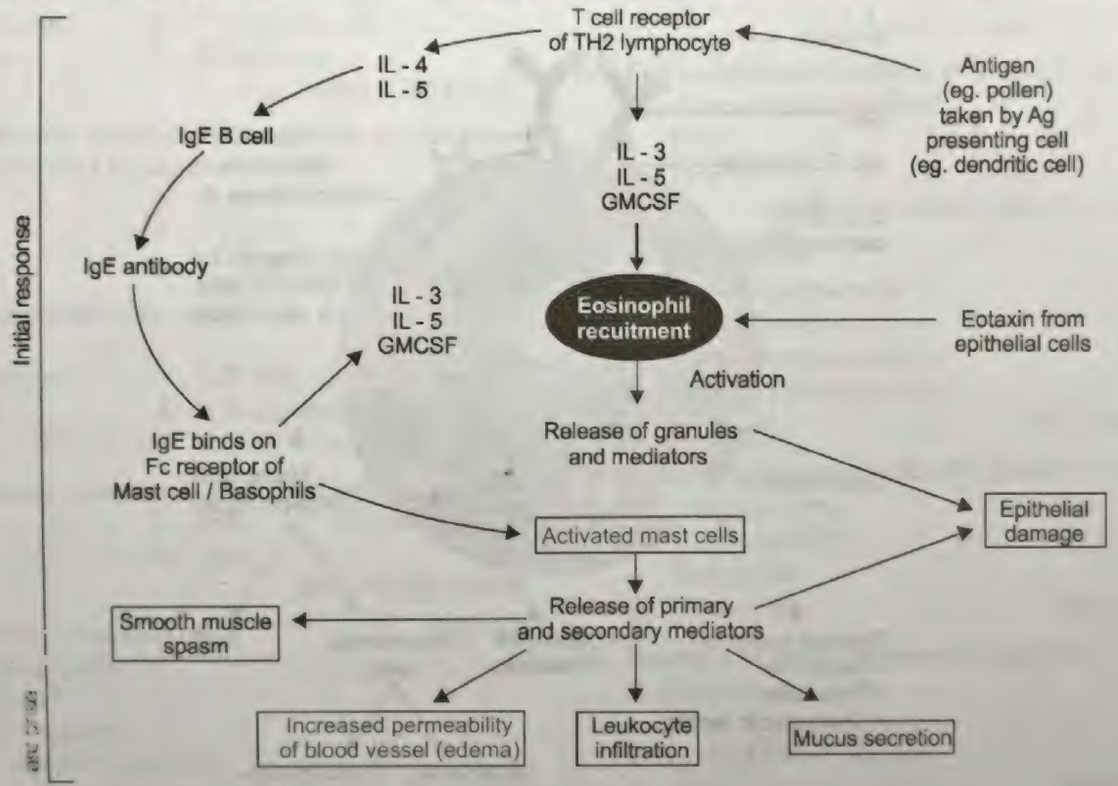
7. **Ans. (d) Schick test** *Ref. Ananthanarayan 7/e, p 103, 8/e, p 111*



- Bacterial exotoxin can be neutralized (e.g. diphtheria, tetanus) while endotoxins can't be neutralized by antitoxin.

8. **Ans. (a) Autoimmune hemolytic anemia**
HSN involving blood components are usually Type II HSN. [It is not the rule, it is just for remembering]

9. **Ans. (a), (b), (c) and (d) Type I reaction Large amount of histamine released Cytokines like IL-4, IL-5, IL-6, GMCSF are released and Mediated through allergen specific IgE** *Ref. Robbin's 8/e, p 19*



10. **Ans. (d), i.e. Wiskott-Aldrich syndrome** *Ref. Ananthanarayan 9/e, p 172*

Disorders of phagocytosis:

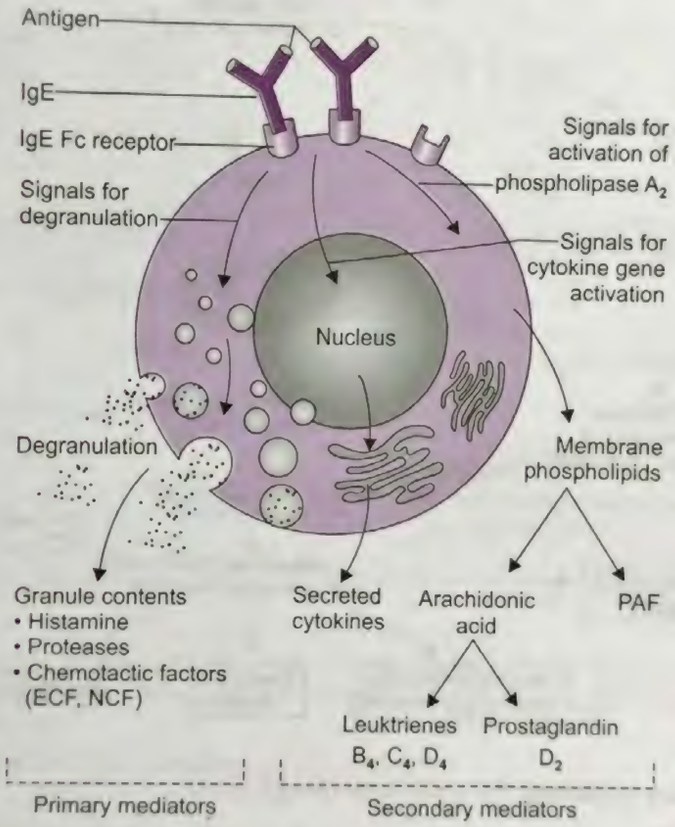
- Chronic granulomatous disease
- Myeloperoxidase deficiency
- Chediak-Higashi syndrome
- Leukocyte G6PD deficiency
- Job's syndrome
- Tuftsin deficiency
- Lazy leukocyte syndrome
- Hyper-IgE syndrome
- Actin binding protein deficiency
- Shwachman's disease

11. Ans. (a, b, c, d), i.e. All options *Ref. Ananthanarayan 9/e, p 164; Robbins 7/e, p 209*

Mediators of Anaphylactic Reaction

a. **Primary mediators** (These are present in mast cell granules) Includes:
   - Biogenic Amines : Histamine, 5 Hydroxytryptamine
   - Enzymes : Neutral protease (Chymase, tryptase) and several acid hydrolases
   - Proteoglycans : Heparin

b. **Secondary mediators:**
   - Leukotrienes : LTC4, D4, B4
   - Prostaglandin D_2
   - Platelet activating factor
   - Cytokines : IL1, IL3, IL-4, IL-5, IL-6 TNF and GMCSF



Mediators of Anaphylactic Reaction

Chapter Review

1. **An example of type-I hypersensitivity reaction is:** [AI 92]
   a. Schicks test
   b. Mantoux test
   c. Lepromin test
   d. Casoni's test
   [Ref. Paniker 6/e, p 154]

2. **Delayed hypersensitivity involves:** [AIIMS 92]
   a. Neutrophils
   b. Monocytes
   c. Eosinophils
   d. Lymphocytes
   [Ref. Robbin's 7/e, p 206]

3. **Delayed tuberculin test response is due to:** [TN 01]
   a. B lymphocytes
   b. T lymphocytes
   c. Monocytes
   d. Histiocytes
   [Ref. Robbin's 7/e, p 217]

4. **All the following types of hypersensitivity reactions can be demonstrated by skin test except:** [UP 04]
   a. Type I
   b. Type II
   c. Type III
   d. Type IV
   [Ref. Robbin's 7/e, p 206]

5. **Delayed hypersensitivity reaction is mediated by the following:** [Kar 02]
   a. B lymphocytes
   b. NK cells
   c. Mast cells
   d. T lymphocytes
   [Ref. Ananthanarayan 8/e, p 168, 9/e, p 167]

6. **Primary immune response is mediated by:**
   a. IgE
   b. IgM [Jharkhand 04]
   c. IgA
   d. IgD
   [Ref. AA 9/e, p 146]

7. **Tuberculin test is reaction of:** [UP 06]
   a. Anaphylaxis mediated
   b. Cell mediated
   c. Antibody mediated
   d. Immuno complex mediated
   [Ref. Ananthanarayan 9/e, p 167, 8/e, p 168]

8. **All are type-II hypersensitivity reaction except:** [Jharkhand 06]
   a. Hemorrhagic disease of newborn
   b. Grave's disease
   c. Autoimmune diseases
   d. Hemolytic anemia
   [Ref. Ananthanarayan 9/e, p 166, 8/e, p 165]

9. **Type I hypersensitivity, the mediator is:** [UP 2007]
   a. IgE
   b. Ig E
   c. IgM
   d. IgC
   [Ref. Ananthanarayan 8/e, p 163, 9/e, p 163]

10. **Erythroblastosis fetalis is an example of which type of hypersensitivity reaction?**
    a. Type I
    b. Type II
    c. Type III
    d. Type IV
    [Ref. Ananthanarayan 8/e, p 163, 9/e, p 166]

11. **Casoni's test is:** [Delhi 2006]
    a. Type 1 hypersensitivity
    b. Type 2 hypersensitivity
    c. Type 3 hypersensitivity
    d. Type 4 hypersensitivity
    [Ref. Paniker 6/e, p 154]

12. **Which is concerned with cell mediated immunity?**
    a. B-lymphocyte [DNB 2013]
    b. T-lymphocyte
    c. Eosinophils
    d. Monocytes *[Ref. AA, 9/e, p 152]*

13. **Example of type 4 hypersensitvity is:**
    a. Serum sickness [DNB 2013]
    b. Granulomatous reaction
    c. Schwartzman reaction
    d. Arthus reaction *[Ref. AA, 9/e, p 167]*

Answers 1. d. Casoni's 2. d. Lymphocytes 3. b. T lymphocytes 4. b. Type II 5. d. T lymphocytes
6. b. IgM 7. c. Antibody ... 8. a. Hemorrhagic ... 9. a. IgE 10. b. Type II
11. a. Type 1 12. b. T-lymphocyte 13. b. Granulomatous...

NEET Pattern Questions

1. **Type IV hypersensitivity includes all, except:**
   a. Paul Bunnell test
   b. Lepromin test
   c. Tuberculin test
   d. Granulomatous reactions

   [Ref. Ananthanarayan 9/e, p 167]

2. **Wheal and flare reaction is what type of hyperstensitivity reaction?**
   a. Type I
   b. Type II
   c. Type III
   d. Type IV

   [Ref. Robbins 8/e, p 128]

3. **Type I hypersensitivity is mediated by which of the following immunoglobulins?**
   a. IgA
   b. IgG
   c. IgM
   d. IgE

   [Ref. A.A 9/e, p 163]

4. **Frie test which type of hypersensitivity:**
   a. Type I
   b. Type II
   c. Type III
   d. Type IV

   [Ref. A.A 9/e, p 419]

Frei test:

Demonstration of hypersensitivity by skin testing in a patient of lymphogranuloma venereum. In this test a heat inactivated LGV 0.1 ml is infected intradermally on the forearm. After 48-72 hour an inflammatory nodule > 60 mm diameter suggests positive result.

Answers 1. a. Paul Bunnell test 2. a. Type I 3. d. IgE 4. d. Type IV

Section - B

UNIT – VI Miscellaneous

- Miscellaneous

CHAPTER

39

Miscellaneous

Multiple Choice Questions

1. **Which of the following is not transmitted by soil?**
   a. Coccidiodomycosis b. Tetanus [AI 08]
   c. Brucella d. Anthrax

2. **Isolation is not useful for all except:** [AI 08]
   a. Mumps b. Measles
   c. Hepatitis A d. Pneumonic plague

3. **Congenital infection affecting fetus with minimal teratogenic risk is:** [AI 08]
   a. HIV b. Rubella
   c. Varicella d. CMV

4. **Which of the following congenital infection leads to maximum CNS damage in the fetus:** [AI 08]
   a. Rubella and CMV
   b. Rubella and toxoplasmosis
   c. CMV and toxoplasmosis
   d. HIV and CMV

5. **With reference to *Bacteroides fragilis* the following statements are true except:** [AI 07; AIIMS 06, AIIMS 2011, 2012]
   a. *B. fragilis* is the most frequent anaerobe isolated from clinical samples
   b. *B. fragilis* is not uniformly sensitive to metroni-dazole
   c. The lipopolysaccharide formed by *B. fragilis* is structurally and functionally different from the conventional endotoxin
   d. Shock and disseminated intravascular coagu-lation are common in *Bacteroides* bacteremia

6. **Which of the following is least likely to cause infective endocarditis:** [AI 06]
   a. *Staphylococcus albus*
   b. *Streptococcus faecalis*
   c. *Salmonella typhi*
   d. *Pseudomonas aeruginosa*

7. **A 40-year-old woman presented to the gyencologist with complaint of profuse vaginal discharge. There was no discharge from the cervical os on the the speculum examination. The diagnosis of bacterial vaginosis was made based upon all of the following findings on microscopy except:** [AI 06]
   a. Abundance of gram variable coccobacilli
   b. Absence of Lactobacilli
   c. Abundance of polymorphs
   d. Presence of clue cells

8. **Apart from *Escherichia coli*, the other most common organism implicated in acute suppurative bacterial peritonitis is:** [AI 06]
   a. *Bacteroides*
   b. *Klebsiella*
   c. *Peptostreptococcus*
   d. *Pseudomonas*

9. **All cause malabsorption syndrome except:** [AIIMS May 09, May 10]
   a. Giardiasis b. *Ascaris*
   c. *Strongyloides* d. *Capillaria phillipinesis*

10. **All of the following organisms are known to survive intracellularly except:** [AI 05]
    a. *Neisseria meningitides*
    b. *Salmonella typhi*
    c. *Streptococcus pyogenes*
    d. *Legionella pneumophilia*

11. **Virus can be isolated from clinical samples by cultivation in the following except:** [AI 05]
    a. Tissue culture
    b. Embryonated eggs
    c. Animals
    d. Chemically defined media

12. **It is true regarding the normal microbial flora present on the skin and mucous membranes that:** [AI 05]
    a. It cannot be eradicated by antimicrobial agents
    b. It is absent in the stomach due to acidic pH
    c. It establishes in the body only after the neonatal period
    d. The flora in the small bronchi is similar to that of the trachea

13. **The most common pathogens responsible for nosocomial pneumonia in the ICU:** [AI 05]
    a. Gram +ve organism
    b. Gram -ve organism
    c. Mycoplasma
    d. Virus infections

14. **Which of the following toxins acts by inhibiting protein synthesis:** [AI 04]
    a. Cholera toxin
    b. Shiga toxin
    c. Pertusis toxin
    d. LT of enterotoxigenic *E. coli*

15. **Toxins are implicated the major pathogenic mechanism in all of the following bacterial diarrheas except:** [AI 04]
    a. *Vibrio cholerae*
    b. *Shigella sp.*
    c. *Vibrio parahemolyticus*
    d. *Staphylococcus aureus*

16. **All of the following infections are often associated with acute intravascular hemolysis except:** [AI 03; AIIMS 03]
    a. *Clostridium tetani*
    b. *Bartonella bacilliformis*
    c. *Plasmodium falciparum*
    d. *Babesia microti*

17. **Which of the following are bacteria?** [PGI June 09]
    a. Bacteriophage
    b. Chlamydia
    c. Mycoplasma
    d. Spirochete

18. **Heat stable enterotoxin causing food poisoning is caused by all the following except:** [AI 02]
    a. *Bacillus cereus*
    b. *Yersinia enterocolitica*
    c. *Staphylococcus*
    d. *Clostridium perfringens*

19. **All of the following are sexually transmitted except:** [AI 02]
    a. *Candida albicans*
    b. *Echinococcus granulosus*
    c. *Molluscum contagiosum*
    d. Group B *Streptococcus*

20. **All of the following infections may be transmitted via blood transfusion except:** [AI 02]
    a. Parvo B-19
    b. Dengue virus
    c. CMV
    d. Hepatitis G virus

21. **The following diagnostic tests are useful for corresponding purpose except:** [AI 02]
    a. Ziehl-Neelsen staining - Detection of mycobacteria
    b. Immunofluorescence - Detection of Influenza virus
    c. Specific IgM antibodies - Immunity against Rubella
    d. Specific IgM antibodies - Detection of acute infection

22. **Cystine lactose enzyme deficient medium CLED is preferred over McConkey agar in UTI because:** [AI 01]
    a. Former prevents swarming of *Proteus*
    b. Is a selective medium
    c. Promotes growth of *Pseudomonas*
    d. Promotes growth of Candida and *Staphylococcus*

23. **All of the following bacteria test "Urease positive" except:** [AI 98]
    a. *E. coli*
    b. *Proteus*
    c. *Klebsella*
    d. *Staphylococcus*

24. **Plaque formation in virus is done for:** [AI 98]
    a. Isolation and typing of viurses
    b. Cloning separation of specific viruses
    c. Determining infectivity of virus
    d. Assessing multiplication of virus

25. **Normal commensal of skin:** [PGI June 08]
    a. *Staph. aureus*
    b. *Candida albicans*
    c. *Bacteroides fragilis*
    d. Propiobacterium
    e. *Corynebacterium*

26. **Most common agents responsible, for human, bite infections are:** [AI 98]
    a. Gram –ve bacilli
    b. Gram +ve bacilli
    c. Spirochaete
    d. Anaerobic streptococci

27. **Which bacteria acts by inhibiting protein synthesis:** [AI 98]
    a. *Pseudomonas*
    b. *Staphylococcus*
    c. *Streptococcus*
    d. *Klebsiella*

28. **Prokaryotes are characterized by:** [AI 97]
    a. Absence of nuclear membrane
    b. Presence of microvilli on its surface
    c. Presence of smooth endoplasmic reticulum
    d. All of the above

29. **A substance, when added to a culture causes inhibition of multiplication but on removal causes enhanced growth. This substance is called:**
    a. Bacteriostatic
    b. Bactericidal [AI 96]
    c. Sterilization
    d. Bacteriophage

30. **Reactive arthritis is caused by:** [AIIMS 08]
    a. *Staphylococcus*
    b. *H. influenzae*
    c. *N. gonorrhoe*
    d. *C. trachomatis*

31. **HACEK group includes all of the following except:** [AIIMS 08]
    a. *Hemophilus arophilus*
    b. *Acinetobacter baumanni*
    c. *Eikenella corrodens*
    d. *Cardiobacterium hominis*

32. **Cy Bromide green dye is used for:** [AIIMS 06]
    a. HLPR
    b. PCR
    c. ELISA
    d. Immunofluorescence

33. **The single most common cause of pyrexia of unknown origin is:** [AIIMS 06, 03]
    a. *Mycobacterium tuberculosis*
    b. *Salmonella typhi*
    c. *Brucella* sp.
    d. *Salmonella paratyphi A*

34. **In the gut, anaerobic bacteria outnumber the aerobes by a ratio of:** [AIIMS 06]
    a. 10: 1
    b. 100: 1
    c. 1000: 1
    d. 10,000: 1

35. **In all of the following diseases chronic carriers are found except:** [AIIMS 06]
    a. Measles
    b. Typhoid
    c. Hepatitis B
    d. Gonorrhea

36. **A 30 year old male present with urethritis. All of the following can be causative agent except:**

a. *N. gonorrhoeae* **[AIIMS 04]**
b. *Chalmydia trachomatis*
c. *Trichomonas vaginalis*
d. *Hemophilus ducreyi*

37. Humoral immunodeficiency is suspected in patient and he is under investigation. Which of the following infections would be consistent with the diagnosis: [AIIMS 04]
a. Giardiasis
b. Pneumocystics carinii pneumonia
c. Recurrent sinusitis
d. Recurrent subcutaneous abscess

38. All the following are common nosocomial infection except: [AIIMS 03]
a. *Staph. aureus* b. *P. aeroginosa*
c. Enterobacteriaceae d. Mycobacterium

39. Latent infection is seen in viral infections except: [PGI Dec 08]
a. HSV-2 b. CMV
c. EBV d. HIV
e. Rotavirus

40. With reference to *Bacteroides fragilis* all of the following statement are true except: [AIIMS 03]
a. It is the most frequent anaerobe isolated from clinical sample
b. It is not uniformly sensitive to metronidazole
c. The lipopolysaccharide formed by *B. fragilis* is structurally and functionally different from conventional endotoxin
d. Shock and DIC are common in bacteremia due to *B. fragilis*

41. A patient present with frontal abscess. Foul smelling. pus is aspirated. Pus shows red. Fluorenscence on ultraviolet examination. The most, likely organism causing the frontal abscess is: [AIIMS 02]
a. *Bacteroides* b. *Peptostreptococcus*
c. *Pseudomonas* d. *Acanthamoeba*

42. In a patient with UTI, CLED (cysteine lactose electrolyte deficient) Media is preferred over MacConkey's media because: [AIIMS 01]
a. It is a differential medium
b. It inhibits swarming of *proteus*
c. Promotes growth of *Pseudomonas*
d. Promotes growth of *S. aureus* and *Candida*

43. Preformed toxin is important in food poisoning due to all except: [AIIMS 01]
a. *S. aureus* b. *Clostridium botulism*
c. ETEC d. *B. cereus*

44. Endotoxin from gram negative organism is: [AIIMS 00]
a. Polysaccharide b. Glycoprotein
c. Lipoprotein d. Lipopolysaccharide

45. Most common tumor caused by virus is:
a. Warts **[AIIMS 97]**
b. Carcinoma cervix
c. Nasopharyngeal carcinoma
d. Lymphoma

46. Which is not an oncogenic virus: [AIIMS 97]
a. HTLV-1 b. Herpes simplex
c. Papilloma virus d. HBV

47. Viral plaque is made in lab for: [AIIMS 96]
a. Quantitative assay of infectivity of virus
b. Diagnosis of virus
c. Qualitative assay of infectivity of virus
d. Type of virus

48. Exotoxins are: [AIIMS 95]
a. Lipopolysaccharide in nature
b. Produced by gram –ve bacilli
c. Highly antigenic
d. Very stable and resistant to chemical agents

49. The term "viable not cultivable" (VNC) is used for:
a. *M. leprae* b. *M. tuberculosis*
c. *Trepenoma pallidum* d. *Salmonella*
e. *Staph.* **[PGI Dec. 07]**

50. Microorganisms invading the GIT causing gastro-enteritis: [PGI Dec. 07]
a. EHEC
b. *Shigella*
c. *Vibrio parahemolyticus*
d. *Campylobacter*
e. *Salmonella*

51. Which of the following can cause rhabdomyo-lysis? [PGI June 07]
a. *Clostridium perfringens*
b. *Streptococcus*
c. *Clostridium difficle*
d. *Cl. tetani*

52. Genital elephantiasis is seen in: [PGI 06]
a. Donovanosis
b. Lymphogranuloma venereum
c. Congenital syphilis
d. Herpes simplex

53. Rhinosporidiosis is caused by: [PGI 06]
a. Fungus b. Bacteria
c. Virus d. Protozoan
e. Parasite

54. Which human infection spreads through urine: [PGI 06]
a. *Leptospira* b. *Legionella*
c. Plague d. *Diphtheria*

55. Urease test is positive in: [PGI 05]
a. *H. pylori* b. *S. aureus*
c. *Klebsiella* d. *Bacillus cereus*
e. *Pseudomonas*

56. Resolution provided by light microscope is:
a. 200 nm b. 20 nm [PGI 05]
c. 0.2 nm d. 2.0 nm
e. 120 nm

57. Pus cell in diarrhea seen in: [PGI 05]
a. Vibrio cholera b. EPEC
c. Rotavirus d. *Shigella*
e. *Campylobacter*

58. C.M.I is seen in: [PGI 05]
a. Histoplasmosis b. Leprosy
c. Tetanus d. Measles

59. Which of the following has malignant potential:
a. HSV - 1 b. EBV [PGI 05]
c. CMV d. Varicella

60. Man is intermediate host in: [PGI 05]
a. *Taenia saginata* b. *Trichenella spiralis*
c. *Strongyloidis* d. *P. falciparum*

61. Capsulated organism: [PGI 03]
a. *Candida* b. *Klebsiella*
c. *Proteus* d. *Cryptococcus*
e. *Histoplasma*

62. Which of the following are transfusion transmitted viruses: [PGI 03]
a. Hepatitis B b. CMV
c. HTLV - 1 d. Rubella
e. HHV - 8

63. Enteropathogenic organisms are: [PGI 02]
a. *Cryptococcus*
b. *B. coli*
c. *Microsporidium* species
d. *E. dispar*
e. *Giardia intestinalis*

64. Which of the following is a bacteria taxonomically:
a. *Chlamydia* b. *Rickettsia* [PGI 01]
c. *Mycoplasma* d. Prion
e. Bacteriophage

65. Which of the following is transmitted by blood:
a. *Toxoplasma* b. Syphilis [PGI 01]
c. CMV d. Hepatitis B and C
e. Hepatitis E

66. Stool examination is required for diagnosis of infection with: [PGI 01]
a. *Staph.* food poisoning b. Clostridia
c. Shigella d. *Campylobacter*
e. *E. vermicularis*

67. True about bacteria: [PGI 00]
a. Mitochondria always absent
b. Sterols always present in cell wall
c. Divide by binary fission
d. Can be seen only under electron microscope

68. Which of the following are intracellular: [PGI 00]
a. Viruses b. *Chlamydia*
c. *Mycoplasma* d. *Rickettsia*

69. Treatment of partner is required in all infection except: [PGI 00]
a. *Candida* b. Herpes
c. *Trichomonas* d. *Gardnerella*

70. MC commensal gut flora in adult: [PGI 00]
a. *Lactobacillus* b. *Bacteroides*
c. *E. coli* d. *Klebsiella*

71. Obligatory anaerobes are all except: [PGI 99]
a. *Clostridia botulinum* b. *Eikenella corrodens*
c. *Bacteroides* d. *H. pylori*

72. The difference between gram +ve and gram –ve organism is the gram –ve organism contains:
a. Teichoic acid
b. Muramic acid [PGI 98]
c. N-acetyl neuraminic acid
d. Aromatic amino acids

73. Maternal viremia most commonly spreading to fetus in utero: [PGI 98]
a. CMV b. Rubella
c. HIV d. Herpes

74. Common natural flora of skin are: [PGI Dec 08]
a. *Streptococcus* b. *Staphylococcus aureus*
c. *Diphtheroid* d. *E. coli*
e. Enterococci

75. One virus particles prevents multiplication of 2nd virus. This phenomena is: [PGI 97, 96]
a. Viral interference b. Mutation
c. Supervision d. Permutation

76. DNA covering material in a virus is called as:
a. Capsomere b. Capsid [PGI 96]
c. Nucleocapsid d. Envelope

77. The virus causing gastroenteritis are: [PGI Dec 08]
a. Rotavirus b. Norwalk virus
c. Adenovirus d. Hepatadenovirus
e. Enterovirus

78. A male patient presented with granulomatous penile ulcer. On Wright geimsa stain tiny organisms of 2 microns within macrohages seen. What is the causative organism? [AIIMS May 10]
a. LGV
b. Calymmatobacterium granulomatis
c. Neisseria
d. Staph aureus

79. Which ones can be easily cultured from CSF?
a. Polio b. Coxsackie [PGI 09]
c. Echo d. Mumps
e. Rubella

80. **Which of the following does not have non human reservoirs:** [PGI 09]
a. Polio
b. Pertussis
c. Salmonella Typhi
d. Neisseria
e. Cl. Tetani

81. **Which of the following is best stain for fungus:** [AIIMS Nov 09]
a. Muciramine
b. Methenamine silver
c. Alcian blue
d. Hematoxylin and eosin

82. **A patient with history of discharge from right ear for past 1 year presented with severe ear ache. The discharge was cultured and the organism was found to be gram positive cocci. The least likely cause is?** [AI 2011]
a. Pseudomonas
b. Streptococcus pneumoniae
c. Staphylococcus
d. Haemophilus influenza

83. **Most common cause of meningo encephalitis in children is:** [AI 2011]
a. Arbo virus
b. Entero virus
c. HSV
d. Polio virus

84. **Correct Combination of incubation period is:** [PGI 2011]
a. Sypillis : 9-90 days
b. Herpes genitatis : 4-5 week
c. LGV : 3 day-6 week
d. Donovanosis : 1-4 week
e. Chancroid : 2-3 week

85. **With reference to antibiotic resistance all of following statements are true except:** [AI 12, AIIMS 11, May12]
a. The most common mechanism is production of neutralizing enzymes by bacteria
b. Plasmid mediated resistance is exclusively transferred vertically
c. Complete elimination of target is the mechanism by which enterococci develop resistance to vancomycin
d. Alteration of target leisons leads to development of resistance in pneumococci

86. **Which organism cannot be cultured in cell free media:** [PGI 2011]
a. Klebsiella rhinoscleromatis
b. Klebsiella ozaenae
c. Treponema pallidum
d. Pnemocystis jiroveci
e. Rhinosporidium seeberi

87. **The endotoxin of the following gram negative bacteria doesn't play any part in the pathogenesis of the natural disease.** [AIIMS Nov 2012]
a. E. coli
b. Klebsiella
c. Vibrio cholerae
d. Pseudomonas

88. **Which of the following is not a common case of neonatal sepsis in India:** [AIIMS Nov 2013]
a. Staphylococci
b. Klebsiela
c. E.coli
d. Group B Streptococci

89. **Common stain for fungal hyphae:** [AIIMS MAY 2013]
a. Methylene blue
b. Gomori Methanamine silver
c. Congo red
d. Oil red O

90. **Real Time PCR is used for:** [AIIMS May 2013]
a. Multiplication of RNA
b. Multiplication of specific segments of DNA
c. Multiplication of Proteins
d. To know how much amplification of DNA has occurred

91. **True about mechanism of bacterial toxins:**
a. Cholera toxin acts by inhibition of guanyl cyclase
b. Botulinum toxin inhibits Ach release
c. Shiga toxin of shigella dysenteriae act by inhibiting protein synthesis [PGI May 2013]
d. Diphtheria toxin act by inhibiting protein synthesis

92. **All vaccines developed from embryonated eggs except:** [PGI May 2013]
a. Influenza
b. Hepatitis A
c. Yellow fever
d. Rabies
e. CMV

Explanations and References with Illustrative Answers

1. **Ans. (c) *Brucella*** *Ref. Park 22/e, p 265*

Modes of transmission of Brucella:

- *Contact infection (MC)*: Through direct contact with infected tissue, blood, urine, vaginal discharge. Mostly occupational.
- *Food borne infection*: Through ingestion of raw milk, dairy products.
- *Air borne infection*: In the environment of slaughter house.

Infection transmitted through soil:

- Man-soil-man–All the disease transmitted fecorally, e.g. typhoid, hepatitis A, etc.
- Soil as storehouse of spores–tetanus, mycosis, botulism.

2. **Ans. (d) Pneumonic plague** *Ref. Park 27/e, p 112*

Periods of isolation recommended	
Disease	**Duration of isolation**
Chickenpox	Until all lesions crusted; usually about 6 days after onset of rash
Measles	From the onset of catarrhal stage through 3rd day of rash
German measles	None, except that women in the first trimester or sexually active, non-immune women in child-bearing years not using contraceptive measures should not be exposed
Cholera, Diphtheria	3 days after tetracyclines started, until 48 hours of antibiotics (or negative cultures after treatment)
Shigellosis	Until 3 consecutive negative stool cultures
Salmonellosis	
Hepatitis A	3 weeks
Influenza	3 days after onset
Polio	2 weeks adult, 6 weeks pediatric
Tuberculosis (sputum +)	Until 3 weeks of effective chemotherapy
Herpes zoster	6 days after onset of rash
Mumps	Until swelling subsides
Pertussis	4 weeks or until paroxysms cease
Meningococcal meningitis	
Streptococcal pharyngitis	Until the first 6 hours of effective antibiotic therapy completed

3. **Ans. (a) HIV** *Ref. Dutta 6/e, p 301*

HIV has got no teratogenic effect on fetus.

Viral infection in pregnancy:

Infection	Fetal effect
Rubella	Sensoneural deafness
Varicella	Septal defect, PDA, cataract, retinopathy
CMV	Hypoplasia of limbs, limb deformity, choroidoretinal scarring, cataract, microcephaly
Parvo virus	IUGR, microcephaly, Intracranial calcification, Mental retardation, choriodoretinitis, deafness
Mumps	Aplastric crisic, CHF, hydrops
HIV	No ill effect on fetus

4. **Ans. (c) Rubella and toxoplasmosis** *Ref. Dutta 6/e, p 296, 299*

- Among Rubella and CMV, rubella is mainly associated with cardiac anomalies while CMV is associated with CNS anomalies.
- Toxoplasmosis leads to hydrocephalus, choriodoretinitis, cerebral calicfication, microcephaly and mental retardation.

5. **Ans. (d) Shock and disseminated intravascular coagulation are common in Bacteroides bacteremia** *Ref. Ananthanarayan 8/e, p 267 - 268, 9/e, p 269*

Anaerobic Gram negative bacilli. includes Bacteroides, Fusobacterium, Leptotrichia, Prophyromonas, Prevatella.

Bacteroides

MC anaerobe isolated from clinical specimen.

- They are Non-sporing, Non-motile, strict anaerobes and capsulated (Virulence factor).
- They are classified on ***the basis of colonial, biochemical features (Sacchrolytic effects) and on characteristics of short chain fatty acid patterns in gas liquid chromatography.***
- *MC* isolate of *Bacteroides* is ***B. fragilis.***
- They grows well on media such as brain heart infusion agar in an anaerobic atmosphere containing 10% Co_2.
- *B. fragilis* (also *Prevotella melaninogenic*) possess ***lipopolysaccharides (endotoxin)*** that are **less *biologically potent than endotoxins associated with aerobic gram negative bacteria.*** Due to this relative biologic inactivity, infection caused by *Bacteroides* less frequently ***produce the clinical signs of sepsis.***
- ***First line therapy*** for anaerobes includes Metronidazole, Ticarcillin/Clavulanic acid, Piperacillin/tazobactan, Imipenem.
- Resistance to metronidazole is seen in <2% cases, i.e. not uniformly sensitive. *... Harrison 18/e, p 1338, 19/e, p 1101*

6. **Ans. (c) *Salmonella typhi*** *Ref. Jawetz 25/e, p 748; Mandell's Principal & Practice of Infectious Disease 6/e, p 998; Braunwald's Heart Disease 7/e, p 1637*

Though both salmonella and pseudomonas cause endocarditis, pseudomonas is a more common etiologic agent than salmonella.

Organism causing endocarditis		
• Streptococci ***(MC)*** (60-80%):	– S. viridans (30-40%) – Other streptococci (15-25%)	– Enterococci (5-18%)
• Staphylococci (20-35%):	– Coagulase positive (10-27%)	– CoNS (1-3%)
• Gram negative bacilli (1.5-13%):	– Enterobacteriaceae	– Pseudomonas
• HACEK Group of Organisms		
• Fungi	– Candida	– Cryptococcus

Remeber:

- Among streptococci ***MC*** cause are S. sanguis, S. bovis, S. mutans, S. mitior.
- Among enterobacteriaeeae ***MC*** cause – Salmonella.
- P. aeroginosa is ***MC*** gram negative bacilli causing endocarditis.

7. **Ans. (c) Abundance of polymorphs** *Ref. Shaw's 13/e, p 129; COGDT 10/e, p 670*

Bacterial Vaginosis

- Defined as alteration in normal vaginal flora rather than true infection
- Causative organism:
  - *G. vaginalis*
  - *H. vaginalis*
  - *Mobiluncus*
- **Microscopy of vaginal secretions** in ***bacterial vaginosis*** shows:
  - Characteristic **clue cells**
  - Decreased or absent lactobacillus
  - Decreased leucocytes.
- **Clinical criteria for diagnosis:**
  - Homogenous white non inflammatory discharge with fishy odour.
  - Microscopic presence of >20% clue cells.
  - Vaginal discharge with pH > 4.5
  - Fishy odor with or without addition of 10% KOH.
- **Treatment:** Metronidazole for both pregnant and non-pregnant women.

Remember:

- Clue cells represent epithelial cells adherant to G. vaginalis.
- Bacterial vaginosis is most prevalent vaginal infection.

8. **Ans. (b) *Klebsiella*** *[Ref. CSDT 13/e, p 465; Harrison 18/e, p 1078, 19/e, p 846]*

Causative organism of acute bacterial peritonitis:

Aerobic (30%)	Anaerobic (10%)
– E. coli ***(MC)***	– Bacteroides
– Klebsiella	– Peptostreptococci
– Enterococci	

Remember: In 60% of cases mixed anaerobic and aerobic infection is found.

9. **Ans. (b) *Ascaris*** *Ref. Journal of digestive disease and sciences 53(3) March 2008 672-679*

Parasites causing Malabsorption	
Adults	**Children**
– Giardia lamblia (MC)	– Giardia lamblia (MC)
– E. histolytica/dispar	– Cryptosporidium
– Ankylostoma duodenale	– E. histolytica/dispar
– H. nana	– A. duodenale
– Stronglyloids*	
– Capillaria philipinesis**	

10. **Ans (c) Streptococcus pyogenes** *See below*

Intracellular organisms are:

a. Bacteria	b. Parasites	c. Viruses are obligate intracellular parasite	d. Fungi
• Listeria monocytogens • Legionella • Rickettsia • Mycobacteria TB and mycobacteria leprae • Chlamydia • Neisseria meningococci and Gonococci • Yersinia pestis • Bordetella • Salmonella • Calymmatobacterium Gronunomatis (Donovania) • Shigella • Brucella • Pneumococci	• Babesia • Plasmodium • Cryptosporidium parvum • Microsporidia sp. • Toxoplasma		• Histoplasma capsulatum

Mnemonic: LLRM Medical College Ne Yaha Bulakar, SDS ko Bahut Pareshaan kiya.

Remember: • Cell Mediated Immunity play vital role against these organisms.

11. **Ans. (d) Chemically defined media** *Ref. Ananthanarayan 8/e 450, 9/e, p 451*

Isolation of virus

Method of isolation consists of:

"Inoculation into animals, eggs or tissue culture after the processed to remove bacterial contaminants."

..... Ananthanarayan 8/e, p 450, 9/e, p 451

As many viruses (adenoviruses, enteroviruses) are frequently found in normal individuals, so only recovery of viral agent from patient doesn't prove that it is the causative agent of the patient illness.

Organism not grown in artificial cultural media are:

- Chlamydia
- Pathogenic treponemes
- Viruses.
- Rickettsia
- M. leprae
- Rhinosporidium
- Pneumocystis

Mnemonic: • Rahul Chalo TV Remote Lao Please

2. Ans. (c). It establishes in the body only after the neonatal period *Ref. Jawetz 27/e, p 169-170*

- "Term normal microbial flora" denotes the population of microorganisms that inhabit the skin and mucous membranes of healthy normal persons. They are not essential to life.
- *MC* resident organisms of upper respiratory tract is streptococci of viridans group.
- *MC* resident bacteria of large intestine is bacteroides species.

Lines from Jawetz clears all choice to you -

"Mucus membranes of mouth and pharynx are often sterile at birth, within 4-12 hrs after birth, viridans streptococci become establish as most prominent member of resident flora and remain so for life."

"In the pharynx and trachea, similar flora establish itself whereas few bacteria are found in normal bronchi. Small bronchi and alveoli are normally sterile."

"Stomach acidity keep the number of microorganisms at a minimum (10^3-10^5) unless obstruction at the pylorus favours the proliferation of gram positive cocci and bacilli."

"Antimicrobials drugs taken orally can, in humans, temporilly suppress the drug susceptible components of the fecal flora."

13. Ans. (b) Gram –ve organisms *Ref. Harrison 18/e, p 1115, 2136-2138, 19/e, p 810*

Guys, this is a twisted question, understand the choice clearly.

- *MC* cause of nosocomial pneumonia in **ICU** now is *S. aureus* (Gram +ve)
- After this comes enterobacteriaceae followed by *Pseudomonas aeruginosa* (Gram –ve).
  - But if we take Enterobacteriaceae and *P. aeruginosa* (Gram –ve organism) together they can outnumber *S. aureus* (Gram +ve organism).

So, the answer will be ***Gram –ve organism.***

Note : *Now word nosocomial has been replaced by hospital acquired pneumonia.*

14. Ans. (b) Shiga toxin *Ref. Ananthanarayan 8/e 284, 9/e, p 287; Harrison 17/e, p 963, 18/e, p 1282, 19/e, p 1056*

Shiga toxin, a protein encoded by the iron regulated chromosomal gene. It is composed of two peptide subunits:

- ***A subunit*** is N-glycosidase that hydrolyzes adenine from specific sites of ribosomal RNA of mammalian 60s-ribosomal subunit, irreversibly inhibiting protein synthesis.
- ***B subunit*** recognizes the receptor glycolipid Gp3 on host cells.

Toxin inhibiting protein synthesis

- Diptheria toxin
- Verocytotoxin or Shiga like toxin of *E. coli* 0157
- Pseudomonas toxin
- Shiga toxin of *Sh dysenteriae I.*

15. Ans. (c) Vibrio parahemolyticus *Ref. Harrison 17/e, p 814, 18/e, p 1084, 19/e, p 266*

Non-inflammatory (Enterotoxin) mediated acute diarrhoeas are:

- Vibrio cholera
- *Clostridium perfringens*
- *S. aureus*
- *Plesiomonas shigelloides*
- *Rota virus*
- *Enteric adenoviruses*
- *Cyclospora sp*
- ETEC (LT or ST)
- *Bacillus cereus*
- *Aeromonas hydrophila*
- Norwalk like viruses
- *Girdia lamblia*
- *Cryptosporidium*
- *Microsporidia*

Vibrio parahemolyticus does not produce enterotoxin, it cause enteritis by invasion of intestinal epithelium. *Ananthanarayan 8/e, p 312, 9/e, p 312*

16. **Ans. (a) *Clostridium tetani*** *See below*

Infection associated with severe hemolysis are:

- Bartonella
- *Clostridium welchii*
- Malaria (*Plasmodium falciparum*)
- Bacteremia with *pneumococci, staphylococi, escherichia coli.*
- Babesiosis

17. **Ans. (b, c) and (d) *Chlamydia, Mycoplasma* and Spirochete** *See below*

Bacteriophage are virus infecting bacteria

18. **Ans. (d) *Clostridium perfringens*** *Ref. Ananthanarayan 7/e, p 252, 9/e, p 255; Harrison 18/e, p 1085, 19/e, p 266*

Heat stable enterotoxin:

1. *Staph aureus* enterotoxin.
2. Enterotoxin of *K. pneumoniae.*
3. Emetic type toxin of *B. cereus* (Diarrheal type is LabileToxin).
4. ST of ETEC (LT is same as toxin of V-cholera).
5. *Yersinia enterocolitis* (by some strains).
6. *Clostridium botulism* toxin (Relatively stable).

19. **Ans. (b) *Echinococcus granulosus*** *Ref. Harrison 17/e, p 822, 18/e, p 1095, 19/e, p 869*

Bacteria	Viruses	Other
TRANSMITTED IN ADULTS PREDOMINANTLY BY SEXUAL INTERCOURSE		
Neisseria gonorrhoeae	HIV (types 1 and 2)	*Trichomonas vaginalis*
Chlamydia trachomatis	Human T-cell lymphotropic virus Type I	*Phthirus pubis*
Treponema pallidum	Molluscum contagiosum virus	
Haemophilus ducreyi	Herpes simplex virus type 2	
Calymmatobacterium granulomatis	Human papillomavirus	
Ureaplasma urealyticum	Hepatitis B virus	
SEXUAL TRANSMISSION REPEATEDLY DESCRIBED BUT NOT WELL-DEFINED OR NOT the PREDOMINAT MODE		
Mycoplasma hominis	*Cytomegalovirus*	*Candida albicans*
Mycoplasma genitalium	HTLV - II	*Sarcoptes scabiei*
Gardnerella vaginalis and other	Hepatitis C, D viruses	
Vaginal bacteria	Herpes simplex virus type 1	
Group b Streptococcus	Epstein-Barr virus	
Mobiluncus spp.	Transfusion-transmitted virus	
Helicobacter cinaedi		
Sporothrix fennelliae		

Remember: • E. granulosus spread by feco-oral route when eggs in dog's feces are ingested either by direct contact with infected dogs or by taking vegetable contaminated with dog's feces.

20. **Ans. (b) Dengue virus** *Ref. Harrison 17/e, p 712; 18/e, p 956*

Infectious complications of blood transfusion

Viral infection	– Hepatitis C virus – Hepatitis G virus – TTV and SENV virus – Cytomegalovirus – Parovirus B-09 – Variant Creutzfeilt jakob disease	– Hepatitis B virus – Hepatitis A virus (rarely) – HIV – HTLV type I – West nile virus
Bacterial infection	– Syphilis – Pseudomonas – Lyme disease	– Yersinia – Gram +ve cocci including coagulase negative staphylococci.
Parasites	– Malaria – Trypanosoma cruzi	– Babesia – Toxoplasmosis

..... *Harrison 17/e, p 1305*

21. Ans. (c) Specific IgM antibodies - Immunity against Rubella *Ref. Ananthanarayan 8/e, p 498, 9/e, p 555*

- **Zeihl Nelson Staining** (Acid fast staining) is used for Mycobacteria detection.
- Rapid diagnosis of Influenza is made by demonstration of virus antigen on the surface of nasopharyngeal cells by immunofluorescence.
- IgM antibodies are antibodies of primary response (IgG is antibody of secondary response). So IgM indicates acute or recent infection. Its production signify that immune response of individual is proper but doesn't mean that person has immunity against that infection (e.g. Rubella).

22. Ans. (d) Promotes growth of *Candida* and *Staphylococcus* *Ref. MM 13/e, p 453; Scot Microbiology 9/e, p 81*

CLED = Cystine Lactose Electrolyte deficient agar

- It is primarily is used for isolation and enumeration of bacteria in urine.
- It inhibits swarming of proteus like McConkey agar.
- It is selective and differential media like McConkey agar.
- Both (Mc. Conkey and CLED) show greenish color, matt surface and rough periphery of *Pseudomonas* colonies.
- It has advantage in supporting growth of certain *Staphylococcus*, *Streptococci* and *Candida* strain.

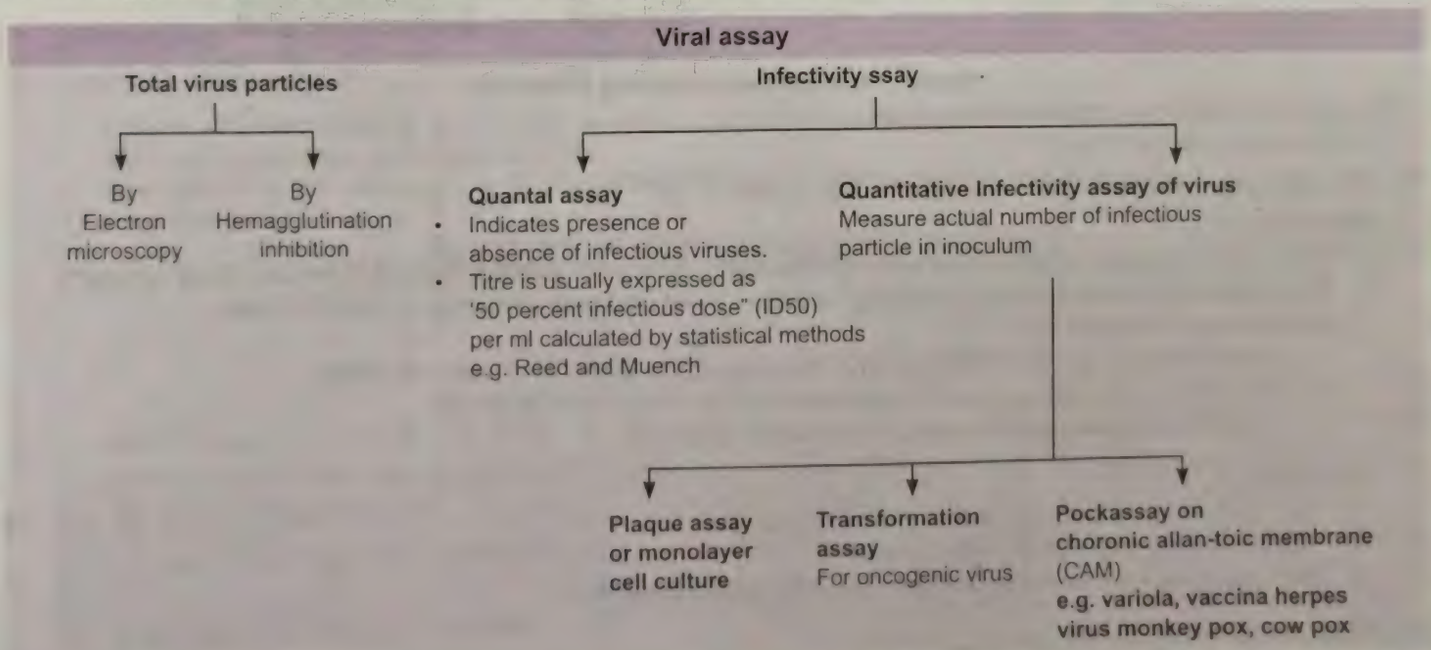
23. Ans. (a) *E. coli* *See below*

Urease test is positive in urease producing bacteria which includes:

i. *Proteus*
ii. *S. aureus*
iii. *Morganella*
iv. *Klebsiella*
v. *Yersinia*
vi. *Cryptococcus*
vii. *Diptheroids*
viii. *Mycobacterium except MAC*
ix. *H. Pylori*

Mnemonic: • PSM KY CD Meri Hai

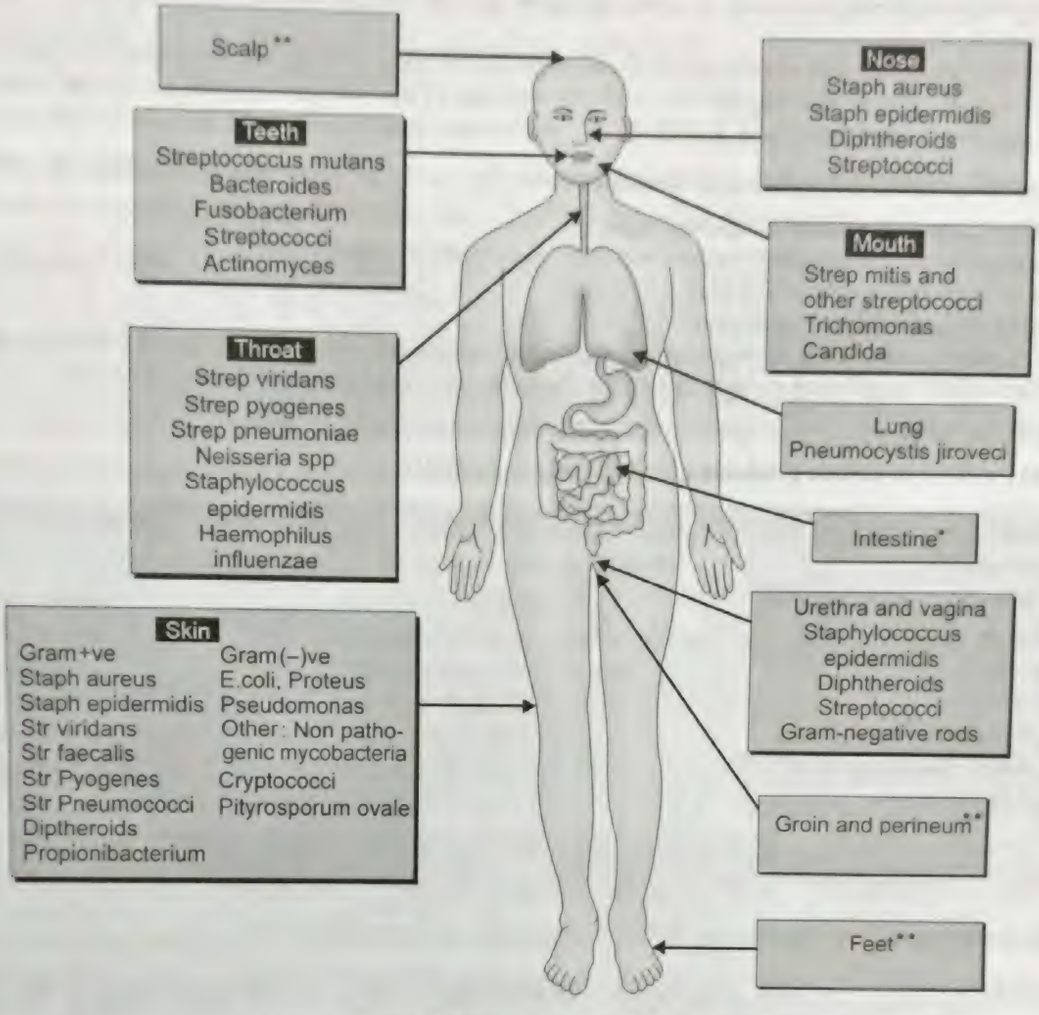
24. Ans. (c) Determining infectivity of virus *Ref. Ananthanarayan 8/e, p 435, 9/e, p 437*



Plaque assay: Each infectious particle give rise to a localized focus of infected cells that can be seen with naked eye. Such foci are knows as plaque and each plaque indicates an infectious virus.

25. **Ans. (a, b, d) and (e) Staph. aureus; Candida albicans; Propiobacterium; Corynebacterium**

Ref. Ananthanarayn 8/e, p 588, 9/e, p 621-623



Normal microbial flora according to locaiton.

*Microbial flora of intestine is described in ans. no. 34.

**Microbial flora is same as of skin

26. **Ans. (d) Anaerobic streptococci** *Ref. CMDT 2014, p 1243*

Human bites

- Human bites are usually inflicted by children; in adults bites are associated with alcohol use and closed fist injury.
- Bites inflicted by children rarely get infected and bites by adults become infected in 15-30% of cases.
- Bacteriology of bite infection:
  - Human bites are mixture of aerobes and anaerobes (54%) or due to aerobes only (44%).
  - *Streptococcus, staphylococcus* and *Eikenella corrodens* are most common aerobes.
  - *Prevotella* and *fusobacterium* are the most common anaerobe

Remember:

- Cat bites are most likely to become infected (30-50%)
- Dog bite become infected only in 5% of cases.
- Pasturella species are the single most common isolate in dog and cat bites.

About the question

This question is of 1998, at that time Harrison 15/e was running. In 15/e, Anaerobes (including pepto streptococcus) were given as more common, So, was the answer.

27. **Ans. (a) *Pseudomonas*** *Ref. Ananthanarayan 8/e, p 284, 9/e, p 316*

Exotoxin A of pseudomonas inhibits protein synthesis.
For details Refer Ans. 14

28. **Ans. (a) Absence of nuclear membrane** *Ref. Ananthanarayan 8/e, p 13, 9/e, p 10*

Differences between prokaryotic and eukaryotic cells

Character	Prokaryotes	Eukaryotes
Nucleus (Main basis of classification)		
• Nuclear membrane	Absent	Present
• Nucleolus	Absent	Present
• Deoxyribonucleoprotein	Absent	Present
• Chromosome	One (Circular)	More than one (linear)
• Mitotic division	Absent	Present
Cytoplasm		
• Cytoplasmic streaming	Absent	Present
• Pinocytosis	Absent	Present
• Mitochondria	Absent	Present
• Lysosomes	Absent	Present
• Golgi apparatus	Absent	Present
• Ribosomes	70s	80s
Chemical composition		
• Sterols	Absent	Present
• Muramic acid	Present	Absent
• Amoeboid movement	+/–	+/-
• Flagella and Pilli	+/–	+/-
• Phosphorylation site	Plasma membrane (Mesosomes)	Ribosomes (Mitochondria)

29. **Ans. (a) Bacteriostatic** *Ref. Jawetz 27/e, p 63, Ananthanarayan 9/e, p 28*

Bacteriostatic – Chemical or substance inhibiting growth without killing.
Bactericidal – Chemical or substance killing organism.
Sterilization – The process by which article or medium is freed of all living microorganism either in the vegetative or spore state.
Bacteriophage – Virus infecting bacteria.

30. **Ans. (d) *C. trachomatis*** *Ref. CMDT 2014, p 825*

- Reactive arthritis (formerly called Reiter syndrome) is the clinical tetrad of uretheritis, conjunctivitis (or uveitis), mucocutaneous lesions and aseptic arthritis.
- Most cases develop within days weeks after either a dysenteric infection or urogential infection.
- Associated infection:

GI	Urogenital
• Shigella • Salmonella • Yersina • Compylobacter	• Chlamydia trachomatus • Ureoplasma urealyticum

- Arthritis is most commonly assymetric, and frequently involves the large joints (most commonly ankle and knee); Sacroiliatis and ankylosing spondylitis is observed in atleast 20% of patients; especially after frequent recurrences.

31. **Ans. (b) *Acinetobacter baumanni*** *Ref. Harrison 17/e, p 926; 18/e, p 1233*

HACEK organism are a group of fastidious, slow growing, Gram negative bacteria whose growth requires an atmosphere of CO_2.

Species belonging to this group include:

- *Hemophilus* species
- *Actinobacillus* actinomycetemcomitans

- *Cardiobacterium hominus*
- *Eikenella corrodens*
- *Kingella kingae*

Endocarditis is the *most common* disease caused by them.

32. Ans. (b) PCR *See below*

Cy Bromide green dye is a dye that binds to minor groove of double stranded DNA and generates fluorescence.

Uses:

- To determine presence of amplified DNA product.
- For optimizing PCR reaction.

33. Ans. (a) *Mycobacterium tuberculosis* *Ref. Harrison 16/e, p 117, 18/e, p 159, 19/e, p 136*

Fever of unknown origin

Defined as:
- Temperature > 38.3°C (> 101°F) on several occasions
- Duration of fever > 3 weeks
- Failure to reach diagnosis despite 1 week of inpatient investigations

CAUSES				
Infections (13%)	**Neoplasm (7%)**	**Non-infectious inflammatory (22%)**	**Miscellaneous (7%)**	**Undiagnosed (51%)**
– *Mycobacterium TB* – Abdominal abscess – Endocarditis – UTI – Viral infections: CMV EBV – Kala azar – Brucellosis	– Lymphoma – Leukemia – Solid tumours			

Infections such as extrapulmonary TB and in endemic areas: typhoid fever and malaria remain a leading diagnosable cause of FUO.

34. Ans. (c) 1000:1 *Ref. Jawetz 25/e, p 162, 27/e, p 169*

Anaerobes outnumber facultative organism by 1000 fold.

Normal flora of intestinal tract

- At ***birth*** the intestine is sterile, but organism are soon introduced after birth.
- In breastfeed children lactic acid streptococci and lactobacilli seen.
- Microorganism are ***minimum*** (10^3-10^5g/ of contents) in stomach.
- In ***upper intestine*** lactobacilli and enterococci predominate.
- In ***colon*** *Bacteroides fragilis* is the *most common* organism found.
- Intestinal bacteria are important in synthesis of vitamin K.

35. Ans. (a) Measles *Ref. Park 22/e, p 154*

In measles and whooping cough only cases are found with no carriers.

Chronic carriers seen in:

- Typhoid
- Dysentery
- Hepatitis B
- Cerebrospinal meningitis
- Malaria
- Gonorrhea

36. Ans. (d) *Hemophilus ducreyi* *Ref. Ananthanarayan 8/e 231; Harrison 18/e, p 1097, 19/e, p 870*

Causes of urethritis are:	
— *Neisseria gonorrhoea* (**MC**)	— *Chlamydia trachomatis*
— *Ureoplasma urealyticun*	— *Mycoplasma hominis*
— *Herpes virus*	— *Cytomegalovirus*
— *Gardenella vaginalis*	— *Acinetobacter woffi, Ac calcoaceticus*
— *Candida albicans*	— *Trichomonas vaginalis*

37. Ans. (b) *Pneumocystis carinii* pneumonia *See below*

Infection in patients with defects in humoral immunity:

- Recurrent or chronic sinopulmonary infection otitis media, meningitis and bacteremia; *MC* by pyogenic bacteria such as *H. influenzae; Strep pneumoniae; Staphylococci*
- Response to viral infection is good except increased risk of:
  - HBV
  - Polio
  - Echoviruses
  - Adenoviruses
- **Parasitic** – Giardial diarrhea
- Less frequent – Bacterial infection of skin and urinary tract.

Infection in patient with deficient cell mediated immunity:

- Disseminates virus infection of Herpes simplex, Varicella zoster, and CMV
- *Mucocutaneous candidiasis* – Almost invariably
- Pneumonia caused by *P. carinii*
- Severe Enteritis caused by Cryptosporidium
- *T cell deficiency is always accompanied by some abnormality of antibody response so patient with T cell defect are also subject to overwhelming bacterial infection.*

38. Ans. (d) *Mycobacterium* *Ref. Harrison 17/e, p 835-838, 18/e, p 1114-1116, 19/e, p 913*

- **Most important group of hospital pathogens are:**
  i. Enteric gram negative bacilli:
     - *E. coli*
     - *Klebsiella*
     - *Enterobacter*
     - *Proteus*
     - *Serratia*
  ii. *S. aureus*
  iii. *Pseudomonas aeruginosa* and other pseudomonas
  iv. Tetanus spores
  v. Yeast (*Candida albicans*), moulds (*Aspergillus mucor*)
  vi. Protozoa (*E. histolytica, Plasmodia, P. carinii, T. gondii*)

Nosocomial infection	Most common causative organism
• Urinary tract infection (**MC Nosocomial infection**)	*E. coli, Candida*
• *Early onset pneumonia* (within 4 days)	*Strept pneumoniae*
• *Late onset pneumonia*	*S. aureus, P. aeruginosa*
• *Surgical wound infections*	***S.aureus***, coagulase negative staphylococcus
• *Infections related to vascular acess*	**Coagulase negative Staph**, *S. aureus*

Remember:

- ***Candida* is now the MC pathogen in nosocomial UTI in ICU patients.** ..... *Harrison 19/e, p 914*
- Examples of some emerging and potential, epidemic problems in hospitals are: Chickenpox TB, Group A streptococci, Aspergillus, Legionella

39. Ans. (e) Rotavirus *Ref. Harrison 17/e, p 1084, 18/e, p 1590, Ananthanarayan 7/e, p 450, 9/e, p 445*

Latent Infection

- Infection in which the infectious agents lies dormant within the host without *symptoms (often without detectable presence in blood, tissue* or other secretion) and without release from host.

Virus causing latent infection		
– HSV 1 and 2	– EBV	– CMV
– VZV	– HHV-6	– HHV-7
– HHV-8	– HIV	– Human papilloma virus
– Prions	– Slow virus infection	– Oncogenic virus

40. Ans. (d) Shock and DIC are common in bacteremia due to *B. fragilis* *Ref. Ananthanarayan 8/e, p 267, 9/e, p 269*

***Already explained, refer Ans.* 5**

41. Ans. (a) *Bacteroides* *Ref. Ananthanarayan 8/e 267, 9/e, p 269; Jawetz 25/e, p 274*

This is a case of brain abscess secondary to *P. melaninogenicus* (formerly called as *Bacteroides melaningenicus*)

- *Provotella includes anaerobic gram negative bacilli* which are inhibited by 20% bile.
- ***MC*** isolate is ***P. melaninogenicus*** (Previously called *Bacteroides melaningenicus*)
  - *P. melaninogenica forms black or brown* ***colour*** *colonies*
  - *Colony colour is not due to melanin but due to a hemin derivative*
  - ***Cultures of*** *P. melaninogenica and even dressings from wounds infected with the bacillus give characteristic red fluorescence* **when** exposed to ultraviolet light.

Remember: Anaerobes causing brain absecess – B. fragilis, Peptostreptococcus, Provotella etc.

42. Ans. (d) Promotes growth of *S. aureus* and *Candida* *Ref. MM 13/e, p 453; Scot Microbiology 9/e, p 81*

***Already explained, refer Ans.* 22**

43. Ans. (c) ETEC *Ref. Ananthanarayan 8/e, p 198, 263, 247, 9/e, p 279*

Preformed toxins have role in:

- Staphylococcal food poisoning (enterotoxin)
- Botulism food poisoning
- Emetic type of food poisoning of *B. cereus* (resemble staph enterotoxin)

In case of **preformed toxin, incubation period is 'short'** in comparison to when organism has to produce toxin in the intestine.

44. Ans. (d) Lipopolysaccharide *Ref. Ananthanarayan 8/e, p 78, Table (9.1), 9/e, p 75*

Distinguishing features of exotoxins and endotoxins

Exotoxins	Endotoxins
• Proteins	Lipopolysaccharides
• Heat labile	Heat stable
• Actively secreted by cells; diffuse into surrounding medium	Form part of cell wall; do not diffuse into surrounding medium
• Readily separable from cultures by physical means such as filtration	Obtained only by cell lysis
• Action usually enzymic	No enzymic action
• Specific pharmacological effects for each exotoxin	Effect nonspecific; action common to all endotoxins
• Specific tissue affinities	No specific tissue affinity
• Active in very minute doses	Active only in very large doses
• Highly antigenic	Weakly antigenic
• Action specifically neutralized by antibody	Neutralization by antibody ineffective
• Can be toxoided	Can't toxoided
• Generally formed by gram positive including some gram negative *shigella, vibrio cholera,* ETEC, *V. parahemolyticus, Aeromonas Y. enterocolitica, P. aeroginosa*	Generally formed by gram negative bacteria

45. Ans. (a) Warts *Ref. Harrison 17/e, p 1117-1118, 18/e, p 1482*

Disease	Associated human papilloma viruses
Common warts (Verruca vulgaris)	**1, 2, 4**, 26, 27, 29, 41, 57, 65, 77
Condyloma accuminatum (Anogenital warts)	**6, 11, 30**, 42, 43, 44, 45, 51, 54
Cervical carcinoma	**16, 18, 31, 33**, 35, 39, 45
Laryngeal papillomas	**6, 11**
Low grade intraepithelial neoplasias	**6, 11, 16, 18**

46. Ans. (b) Herpes simplex *Ref. Jawetz 25/e, p 592, 27/e, p 620*

Association of viruses with human cancers.

Virus family	Virus	Human cancer
Papillomaviridae	Human papilloma viruses	Genital tumors, squamous cell carcinoma, Oropharyngeal carcinoma
Herpesviridae	EB virus	Nasophryngeal carcinoma, African Burkitt's, lymphoma, B cell lymphoma
Hepadna viridae	Hepatitis B virus	Hepatocellular carcinoma
Retroviridae	HTL virus	Adult T cell leukemia
	HIV	AIDS related malignancies
Flaviviridae	Hepatitis C virus	Hepatocellular carcinoma

47. Ans. (a) Quantitative assay of infectivitiy of virus *Ref. Ananthanarayan 8/e 435, 7/e, p 441 - 442*
Already explained, refer Ans. 24

48. Ans. (c) Highly antigenic *Ref. Ananthanarayan 8/e, p 78, 9/e, p 75*
Already explained, refer Ans. 44

49. Ans. (a) and (c) Leprae, *Treponema pallidum*
Already explained, refer Ans. 11

50. Ans. (b, c, d) and (e) Shigella, *Vibrio parahemolyticus*, *Campylobacter* and *Salmonella*

Invasive Diarrhea		
Minimal inflammation	**Variable inflammation**	**Severe**
• Rotavirus • Norwalk agent	• *Salmonella* • *Campylobacter* • *Aeromonas* • *Vibrio parahaemolyticus* • *Yersinia*	• *Shigella* • *EIEC* • *Entamoeba histolytica*

51. Ans. (a, b, d) *Clostridium perfringens*, *Streptococcus* and *Cl. tetani*

Rhabdomyolysis			
Viral causes	**Bacterial causes**		**Fungal causes**
• Influenzae types A and B (most common) • HIV • Ebstein-Barr virus • Echovirus • Cytomegalovirus • Adenovirus • Herpes simplex virus • Parainfluenza virus • Varicella-zoster virus • Coxsackievirus	• *Francisella tularensis* • *Streptococcus pneumoniae* • *Group B streptococci* • *Streptococcus pyogenes* • *Staphylococcus epidermidis* • *Escherichia coli* • *Borrelia burgdorferi* • *Clostridium tetani* • *Viridans streptococci*	• *Rickettsia species* • *Salmonella species* • *Listeria species* • *Legionella species* • *Mycoplasma species* • *Vibrio species* • *Brucella species* • *Bacillus species* • *Leptospira species*	• *Candida* • *Aspergillus*

52. Ans. (a) and (b) Donovanosis and *Lymphogranuloma venerum* *Ref. Ananthanarayan 8/e, p 396, 9/e, p 397*

Genital elephantiasis is seen in Donovanosis which is caused by Klebsiella granulomatis (Calymmatobacterium granulomatosis or Granuloma inguinale/venerum).

Donovanosis

- Chronic progressive bacterial infection of the genital region that is generally sexually transmitted.
- **Causative organism:**
  - ***Klebsiella granulomatis (formerly called** Calymmatobacterium granulomatis*: Gram negative, nonmotile, encapsulated bacterium.
  - Morphologically and antigenically related to Klebsiella.
  - Grow on egg yolk or modified levinthal agar.
- I.P. - 1-4 weeks.
- **Clinical features:**
  - Disease begin as one or more subcutaneous nodules that erode through the skin to produce clean ulcers, (sharply defined) which is usually painless lesion.
  - Genitalia are involved in 90% of cases.
  - Genital swelling particularly of labia is common and may progress to pseudoelephantiasis.
  - Complications include phimosis and paraphimosis.
- **Diagnosis:**
  - Diagnosis is made by demonstration of typical intracellular Donovan bodies (safety pin appearance) in Wright Giemsa stain.
- **Treatment:**
  - Azithromycin - ***DOC.***
  - Doxycycline - Second ***DOC.***

Remember:

- Genital elephantiasis is also seen in lymphatic filariasis. *... Harrison 16/e, p 1260*
- Vulval elephantiasis or esthiomene is seen in lymphogranuloma venerum *... Ananthanarayan 9/e, p 421*

53. Ans. (a) Fungus *Ref. Ananthanarayan 8/e, 609, 9/e, p 603; Dhingra 3/e, p 197*

- Rhinosporidiosis is a chronic granulomatous disease characterized by development of friable polyp usually confined to the nose, mouth or eye.
- Polyp is highly vascular which bleeds easily on touch. Its surface is studded with white dots which represents the sporangia of fungus.
- **Causative agent**
  - *Rhinosporidium seeberi*. The fungus has not been cultivated in media.
  - The infection is supposed to originate from stagnant water or aquatic life.
- **Diagnosis:**
  - Biopsy shows round or oval sporangia filled with spores which may burst through chitinous wall.
- **Treatment:**
  - Complete excision with diathermy knife and cauterization of its base.
  - Dapsone and Amphotericin B are also effective.

54. Ans. (a) Leptospira *Ref. See below*

Disease	Mode of infection
Leptospira	Water contaminated by the urine of carrier animals enter the body through cut or abrasions on the skin or through intact mucosa of mouth, nose or conjunctiva.
Legionella	Inhalation of aerosols produced by AC, cooling towers.
Plague	Bite of rat flea, droplet infection
Diptheria	Droplet infection

55. Ans. (a, b, c) *H. pylori, S. aureus* and *Klebsiella* *Ref. See below*

Already explained, refer Ans. 23

. **Ans. (a) 200 nm** *Ref.See below*

Resolving power are:

Light microscope	–	0.25 μm-0.3 μm	= 200-300 nm
Electron microscope	–	2-10 Å	= 0.2-1 nm

7. **Ans. (d) and (e)** ***Shigella*** **and** ***Campylobacter***

GASTROINTESTINAL PATHOGENS CAUSING ACUTE DIARRHEA

Mechanism	Location	Illness	Stool findings	Examples of pathogens involved
Noninflammatory *(enterotoxin)*	Proximal small bowel	Watery diarrhea	No fecal leukocytes; mild or no increase in fecal lactoferrin	Vibrio cholerae, enterotoxigenic *Escherichia coli* (LT and /or ST), *Clostridium perfringens, Bacillus cereus,S. aureus, shigelloides, rotavirus,* Norwalk-like viruses, enteric adenoviruses, *Giardia lamblia, Cryptosporidium spp., microsporidia*
Inflammatory *(invasion or cytotoxin)*	Colon or distal small bowel	Dysentery or inflammatory diarrhea	Fecal Polymorphonuclear leukocytes; substantial increase in fecal lactoferrin	*Shigella spp., Salmonella spp.,Campylobacter jejuni, enterohemorrhagic E. coli, enteroinvasive E. coli, Yersinia enterocolitica, Vibrio parahaemolyticus, Clostridium difficile, Entamoeba histolytica*
Penetrating	Distal small bowel	Enteric fever	Fecal mononuclear leukocytes	*Salmonella typhi, Y. enterocolitica, Campylobacter fetus*

58. **Ans. (a, b) and (d) Histoplasmosis, Leprosy and Measles**

Already explained, refer Ans. 10

59. **Ans. (b) EBV** *Ref. Jawetz 25/e, p 592, 27/e, p 620*

Already explained, *refer Ans. 46*

60. **Ans. (d)** ***P. falciparum***

Man is intermediate host (Secondary) in:

- *Plasmodium*
- *Toxoplasma gondii*
- *Echinococcus granulosus* [dog tapeworm/hydatid worm/*Taenia echinococcus*]
- *Sarcocystis lindemanni*
- *T. solium* (man also act as definitive host).

61. **Ans. (b) and (d)** ***Klebsiella*** **and** ***Cryptococcus***

Capsulated bacterias are:

- *Pneumonococcus*
- *Kleibsella*
- *Yersinia*
- *N. Meningococci*
- *Vibrio parahemolyticus*
- *Bacillus anthrax*
- *H. influenza*
- *Bordetella*
- *Cl. Perfringe's* and *butyricum*

Mnemonic : PAKIYB - M.C.V

Remember : *Capsulated fungi is Cryptococcus neofomans not Histoplasma capsulatum.*

62. **Ans. (a, b) and (c) Hepatitis B; CMV and HTLV - 1** *Ref. Harrison 17/e, p 712, 18/e, p 856*

Already explained, refer Ans. 20

63. Ans. (b, c, d) and (e) *B. Coli, Microsporidium* species, *E. dispar* and *Giardia intestinalis*

Let consider each options.

a. ***Cryptococcus*** is **fungus** causing pneumonitis, cutaneous infection and cryptococcal meningitis. *... Ananthanarayan 8/e, p 610, 9/e, p 613*

b. ***Balantidum coli*** – **only ciliate** protozoan parasite of man. Its trophozoite lives in ***large intestine.*** *... Paniker 7/e, p 107*

c. ***Microsporidium sp.*** – It ***is intracellular parasite.***
   - Transmission is chiefly by ingestion of spores in food or water.
   - Transplacental transmission is common.
   - Also cause infection in AIDS patient *....Jawetz 27/e, p 723*

d. ***E. dispar***
   - It is protozoa (amoeba)
   - In case of intestinal amoebiasis, parasite remains luminal and trophozoite multiply as a bacteria feeding colony, ultimately encyst and pass out in feces. These are presumed to be due to *E. dispar.*

   It is lumen dwelling non-pathogenic commensal.
   It is distinguished from *E. histolytica* only by isoenzyme electrophoresis and DNA analysis. *... Jawetz 27/e, p 710*

e. ***Giardia lamblia*** is intestinal **flagellates.** *... Paniker 7/e, p 30*

64. Ans. (a, b) and (c) *Chlamydia, Rickettsia* and *Mycoplasma* *Ref. See index of any Microbiology Book*

Prion is proteinaceous infectious particle, without nucleic acid, cause slow virus disease.
Bacteriophage is the virus that infects bacteria.

65. Ans. (a, b) and (c) Toxoplasma, Syphilis and CMV *Ref. Harrison 17/e, p 712, 18/e, p 956*

Already explained, refer Ans. 20

66. Ans. (a, b, c, d) and (e) All are correct options *Ref. Jawetz 25/e, p 720; Harrison 17/e, p 814 Tab. (113-1)*

Following infections required stool examination for diagnosis:

i. Toxins (of *staphylococcus, Clostridia, Vibrios,* Toxigenic *E.coli*)
ii. *Shigella*
iii. *Salmonella*
iv. *Campylobacters*
v. *Yersinia enterocolitica*
vi. *Vibrios*
vii. Enteric bacteria
viii. Enteroviruses
ix. Intestinal parasites (*Giardia, E. histolytica, B. coli, Cryptosporidium, Fasciola hepatica, E. vermicularis* etc).

67. Ans. (a) and (c) Mitochondria always absent and Divide by binary fission *Ref. Ananthanarayan 8/e, p 12, 17, 9/e, p 10*

- Only bacteria having sterol in its cell membrane: **Mycoplasma**
- **Bacteria can be seen by:**
  - Optical or light microscopy
  - Phage contrast microscopy
  - Dark field or dark ground microscopy
  - Electron microscopy.

For details refer ans 28

68. Ans. (a, b) and (d) Viruses, Chlamydia and Rickettsia

Already explained, refer Ans. 10

69. Ans. (d) *Gardnerella* *Ref. Harrison 17/e, p 827, 18/e, p 1100, 19/e, p 295 - 296; Shaws 12/e, p 98 - 100*

"Treatment of male partners with metronidazole does not prevent recurrence of bacterial vaginosis/gardenella associated with vaginal discharge."

Treatment of sexual partners is required in:

- *Candidiasis*
- *Herpes genitalis*
- *Trichomoniasis*
- *Chlamydia*
- *Gonococci*

70. **Ans. (a) and (b) *Lactobacillus* and *Bacteroides*** *Ref. Ananthanarayan 8/e, p 590, 9/e, p 622; Jawetz 27/e, p 170*

Normal flora of intestinal tract:

- At birth, intestine is sterile but organism are soon introduced with food.
- **Stomach's acidity** keep the number of microorganism at a minimum **(10^3-10^5 g of contents) level, as** the pH of intestinal contents becomes alkaline, resident flora gradually increases.
- ***In the upper intestine,*** *lactobacilli* and *enterococci* predominate but in lower ileum and caecum, flora is fecal.
- In duodenum there are 10^3–10^6 bacteria per gram of contents, in the jejunum and ileum 10^5–10^8 bacteria per gram; and in the caecum and transverse colon 10^8–10^{10} bacteria per gram. In the sigmoid colon and rectum, there are 10^{11} bacteria per gram of contents and constitutes 60% of fecal mass
- ***In normal adult colon,*** 96-99% of resident bacterial flora consist of *anaerobes - bacteriodes* sp. especially *B. fragilis, fusobacterium sp;* anaerobic lactobacilli e.g. *bifidobacterium; clostridium* (*C. perfringe's*) and *anaerobic gram positive cocci* (*Peptostreptococcus*).
  - Only 1-4% are facultative aerobes (gram-negative coliform bacteria, enterococci, pseudomonas etc.)

IMPORTANCE

a. **Protection:** Normal resident flora displace and inhibit potential pathogen, indirectly by competing for nutrients and receptors or directly through the production of anti microbial factors such as lactic acid.

b. **Immunity:** Commensal organism are important for the development and function of the mucosal immune system. They induce the secretion of IgA, modulate local T-cell response and cytokine profiles.

c. **Metabolic function:** Intestinal bacteria produce short chain fatty add that control intestinal epithelial cell differentiation. They synthesize vitamin K, biotin and folate and enhance ion absorption.

d. **Anti-cancer:** Certain bacteria metabolize dietary carcinogens.

71. **Ans. (b) and (d) *Eikenella corrodens* and *H. pylori*** *Ref. Ananthanarayan 8/e, p 265, 9/e, p 268*

ANAEROBIC BACTERIA

Cocci

A. *Gram positive*
   a. *Peptostretptococcus*
   b. *Peptococcus*

B. *Gram-negative*
   Veillonella

Bacilli

1. *Endospore forming*
   A. Clostiridia

2. *Nonsporing*
   A. Gram positive:
      a. Eubacterium
      b. *Propionibacterium*
      c. *Lactobacillus*
      d. *Mobiluncus*
      e. *Bifidobacterium*
      f. *Actinomyces*
   B. Gram-negative:
      a. *Bacteroides*
      b. *Provotella*
      c. *Porphyromonas*
      d. *Fusobacterium*
      e. *Leptotrichia*

Spirochetes

A. *Treponema*

B. *Borrelia*

72. **Ans. (d) Aromatic amino acids** *Ref. Ananthanarayan 8/e, p 17, 9/e, p 16*

Cell Wall		
Features	**Gram positive**	**Gram negative bacteria**
Plasmolysis	Late	Early
Thickness (Peptidoglycan)	Thicker	Thinner
Variety of amino acids	Few	Several
Aromatic and sulphur containing amino acids	Absent	Present
Lipids	Absent or scant	Present
Teichoic acid	Present	Absent

73. **Ans. (a) CMV** *Ref. Nelson 17/e, p 568, 623, 626, 631*

Transplacental transmission occurs in:			
Viruses	– Rubella	– CMV	– HSV
	– Varicella zoster virus	– Parvo B - 19	– Coxasackie viruses
	– HIV	– West nile virus	– Enteroviruses
	– HBV and HCV	– Measles	
Bacteria	– Syphilis	– *TB*	– *Brucella*
Parasite	– *Plasmodium*	– *T. cruzi*	– Microsporidia
		– *Toxoplasma*	

74. **Ans. (a, b, c) *streptococcus, Staphylococcus aureus, Diptheroids*** *Ref. Ananthanarayan 8/e, p 588, 9/e, p 621-623*

Already explained, see Ans. 25

75. **Ans. (a) Viral interference** *Ref. Ananthanarayan 8/e, p 438, 9/e, p 439*

Viral interference

"Interference in which infection of a cell by one virus inhibits simultaneous or subsequent infection by another virus."

- ***Most important*** *mediator* of interference is *interferon.*
- Interference produced by destruction of cell receptors is seen *with myxoviruses and enterovirus.*

It is *applied* in the field in *controlling poliomyelitis* outbreaks by introducing into the population, the live attenuated poliovirus vaccine.

Remember: Interference is *Nongenetic interaction.*

76. **Ans. (b) Capsid** *Ref. Ananthanarayan 8/e, p 426, 9/e, p 429*

"Capsid is the protein coat which surrounds nucleic acid (RNA or DNA) of virus."

- Capsid + enclosed nucleic acid is known as nucelocapsid.
- ***Function of capsid is:***
  - To *protect the* nucleic acid from inactivation by nucleases and other deleterious agents in the environment.
  - To *introduce viral genome into host cells* by adsorbing readily to cell surfaces.
- ***On the basis of shape capsid can be:***
  - Icosahedral : Herpes virus, Adenovirus, Poliovirus
  - Helical : Rabies virus
  - Complex : Pox virus

77. **Ans. (a, b, c, e) Rotavirus, Norwalk Virus, Adenovirus, Enterovirus** *See below*

Virus causing diarrhea	
– Adenovirus	– Enterovirus
– Rotavirus (MC)	– Norwalk virus
– Adenovirus (Specially serotype 40 & 41)	– Astrovirus
– Calcivirus	– Enterovirus
– Corona virus	

78. **Ans. (b) Calymmatobacterium granulomatis** *Ref. Ananthanarayan 8/e, p 397, 9/e, p 397*

"In donovanosis bacteria appear as round coccobacilli of 1-2 μm, with in cystic spaces inside large morphonuclear cells. They show bipolar condensation of chromatin, giving a safety pin appearance in stained smears".

For details see Answer No. 52.

79. **Ans. (d) Mumps** *Ref. Jawetz 25/e 719*

Virus can be successfully isolated from the CSF in:
- Mumps virus
- Herpers simplex meningitis
- Enterovirus

80. **Ans. (a; b; c) Polio; Pertussis; Salmonella Typhi** *Ref. Park 22/e, p 154; 185, 213*

Human are the only reservoir for:
- B. pertusis
- V. Cholera
- Enterovirus
- Polio
- Salmonella typhi

81. **Ans. (b) Methenamine silver** *Ref. Ananthanarayan 8/e p 601; DR Arora 3/e 656*

Stains for fungi
- Gomori methenamine silver (GMS) ***(Better contrast)***
- Periodic acid Schiff (PAS)
- Alcian blue
- Gridley fungus (GF)
- Giemsa
- Meyer's mucicarmine

Out of these *GMS* is the *best stain for fungi*. For studying tissue response secondary to fungal infection, *Haematoxylin and eosin (H&E) is best.*

Remember: Commonly used culture media for fungus are:
- Sabouraud's glucose agar
- Corn meal agar
- Czapek - Dox medium

82. **Ans. (d) Hemophilus influenza** *Ref. See below*

H. influenzae is a gram (–)ve coccobacilli

Otitis media	*Etiologic bacteria*
Acute otitis media	Pneumococci > H. influenzae > Moraxella > Streptococcus pyogenes
Chronic otitis media	Staph aureus > Pseudomonas

... Infectious disease by Nonathan 2/e, 856

83. **Ans. (b) i.e. Enterovirus** *Ref. Nelson 18/e, p 2521*

Viral Meningoencephalitis

An acute inflammatory process involving the meninges and to a variable extent brain tissue.

Etiology: Enterovirus (MC), Arbovirus, HSV-1, Mumps (in regions where mumps vaccine is not used), EBV, CMV

Clinical features – Headache (most common), usually frontal or generalized. In adoloscent retrobulbar pain is frequent
– Fever, nausea, vomiting

Lab finding
- CSF shows mild mononuclear pleocytosis with absence of bacteremia
- Detection of viral RNA or DNA by PCR is diagnostic

Treatment – Symptomatic in all cases except in HSV meningoencephalitis where acyclovur is beneficial

Note:
— Enterovolrus is the MC cause of viral meningitis in mumps immunized population
— Enterovirus meningitis is particularly prevalent in infants < 3 months of age. Frequently implicated serotypes include Enterovirus 70 71, coxsackie 4, 6, 7, 9, 11

84. **Ans. (a, c and d)** *Ref. Harrison 17/e, p 832*

Disease	Incubation period
Syphilis	9 – 90 days
Herpes	2 – 7 days
Choncroid	1 –14 days
LGV	3 days – 6 weeks
Donovanosis	1 – 4 weeks

85. Ans. is b i.e. Plasmid mediated resistance is exclusively transferred vertically *Ref: Goodman and Gilman's 12/e, p 1377-78*

- ***Drug resistance is more commonly acquired by horizontal transfer.***
- Horizontal transfers occurs through transformation, transduction conjugation. It largely depends on mobile genetic elements like plasmids, bacteriophages. Other mobile elements like transposons, integrin's also participate in acquiring resistance.
- Once acquired resistance in transmitted vertically to its progeny.

Other options

Option 'a' *...Goodman and Gillman 12/e, p 1541*

- Drug inactivation is a common mechanism of drug resistance. *Example:* Resistance to aminoglycosides and beta lactams are usually secondary to production of aminoglycoside modifying enzymes and beta lactams respectively

Option 'c' *...Goodman and Gillmann 12/e, p 1541*

- Enterococcal resistance to glycopeptides (vancomycin and teicoplanin) is the result of alteration of the D-alanyl-D-alanine target to D-alanyl-D-lactate which bind glycopeptides poorly due to the lack of a critical site for hydrogen bonding.

Option 'd' *...Harrison 18/e, p 1157*

- Penicillin resistance in pneumococci is due to alteration in PBP (penicillin binding protein), which is acquired by transformation through horizontal transfer gene from a related streptococci species.

86. Ans. is (c, d, e) Treponema pallidum, Pnemocystis jiroveci, Rhinosporidium seeberi *Ref Ananthanarayan 8/e, p 278, Harrison 18/e, p 1380*

Klebsiella can be grown very well on ordinary media
For details see Ans 11

87. Ans. is (c) i.e. Vibrio *Ref Ananthanarayan 8/e, p 307, 9/e, p 308*

Lipopolysaccharide O antigen (endotoxin) of V. Cholera plays no role in the pathogenesis of cholera, but is responsible for the immunity induced by killed vaccine.

88. Ans. is d i.e Group B Streptococci *Ref. Arch Dis Child Fetal Neonatal Ed2005;90:F220-FF224*

The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries:

- Overall, Gram negative organisms are more common and are mainly represented by Klebsiella, Escherichia coli, Pseudomonas, and Salmonella.
- Of the Gram positive organisms, Staphylococcus aureus, coagulase negative staphylococci (CONS), Streptococcus pneumoniae, and Streptococcus pyogenes are most commonly isolated.

Further it is stated that

Group B streptococcus (GBS) is generally rare or not seen at all, although maternal rectovaginal carriage rates of GBS may be similar to those recorded in developed countries.

Neonatal Sepsis

- **Neonatal sepsis** specifically refers to the presence of a bacteriamic infection (such as meningitis, pneumonia, pyelonephritis, or gastroenteritis) in the setting of fever in a newborn baby.
- It is divided into two categories:
  - Early Onset Sepsis (EOS): EOS refers to sepsis presenting in the first 7 days of life
  - Late Onset Sepsis (LOS), with LOS referring to presentation of sepsis after 7 days
- *Diagnosis:* Culturing for microorganisms from a sample of CSF, blood or urine, is the gold standard test for definitive diagnosis of neonatal sepsis.

Note: In western countries, Group B streptococci is the most common etiologic organism responsible for neonatal sepsis.

89. Ans is b i.e Gomori Methanamine silver *Ref. Chakraborty 2/e 617*

"Gomori Methenamine Silver (GMS) and Periodic acid-Schiff (PAS) are the two most common stains used to look for fungi in tissues and in cytology specimens."

- The GMS stain is more sensitive than the PAS stain as it stains both viable and non-viable fungal elements (which are refractory to other stains) but it stains inflammatory cells (lysosomes) and tissue reticulin too (in addition to fungi.) PAS staining has the slight advantage that the morphology of the tissue adjacent to the fungi can be better visualized, but this can be addressed using a GMS stain and H and E counterstain (which stains only tissue).

90. Ans. (d) To know how much amplification of DNA has occured *Ref. Jawetz 25/e 714, 27/e, p 124; Greenwood 18/e 79*

Real Time PCR

- It is the molecular detection technique that discriminates real time amplification from conventional PCR assays.
- The real-time polymerase chain reaction (PCR) uses fluorescent reporter molecules to monitor the production of amplification products during each cycle of the PCR reaction.
- This combines the DNA amplification and detection steps into one homogeneous assay and obviates the requirement for gel electrophoresis to detect amplification products.
- Its simplicity, specificity, and sensitivity, together with its, more reliable instrumentation, and improved protocols, has made realtime PCR the benchmark technology for the detection of DNA.
- Real time PCR is extremely useful in medical microbiology, with greatest impact on virology.

91. Ans. (b, c, d) Botulinum toxin inhibits Ach release, Shiga toxin of shigella dysenteriae act by inhibiting protein synthesis, Diphtheria toxin act by inhibiting protein synthesis *Ref. Greenwood 18/e p 165, Ananthanarayan 9/e, p 308, 264*

Bacterial Exotoxins

- These are the diffusible protein secreted into the external medium by the pathogen
- According to the mode of action bacterial exotoxin can be classified into:
  a. Type I (Membrane acting): Toxin bind surface receptors and stimulate transmembrane signals and include the super-antigenic toxins
  b. Type II (Membrane damaging): Toxin directly affect membranes, forming pores or disrupting lipid bilayers
  c. Type III (intracellular effector): Toxins translocate an active enzymatic component into the cell and modify an intracellular target molecule.

Mechanism of Action of Important Toxins		
↑ cAMP	:	Cholera toxin; E. coli heat liable toxin
↓ Acetylcholine release	:	Bolulinum toxin
↓ Protein synthesis	:	Diptheria toxin, Shigella toxin, Exotoxin A of pseudomonas
↑ cGMP	:	Heat stable toxin of E. coli

92. Ans (b, e) Hepatitis A, CMV *See below*

Vaccine	*Source*	*Nature*
Varicella	Tissue culture	Live attenuated
Polio		
– Salk	Tissue culture	Killed
– Sabin	Tissue culture	Live
Influenza		
Inactivated	Allantoic cavity of egg	Killed
Live	Egg	Live
Mumps	Chick embryo fibroblast culture	Live
Measles	Human diploid cells	Live
Rabies	Duck egg	Killed
	Chick embryo	
	Tissue culture	
Hepatitis A	Human diploid cell	Live
Hepatitis B	Genetically engineered Clone of S-gene	–
Rubella	Tissue culture	Live

Chapter Review

1. **Segmented RNA is seen in :** [AI 91]
   a. Rabies virus b. Coxsackie B virus
   c. Influenza virus d. Rabies virus
   [Ref. Ananthanarayan 8/e, p 494, 9/e, 495]

2. **Which of the following is a single stranded DNA :** [AI 91]
   a. Herpes simplex b. Parvo virus
   c. Papavo virus d. Rabies virus
   [Ref. Ananthanarayan 8/e, p 439, 9/e, 554]

3. **All are common organisms causing UTI except :** [AIIMS 92]
   a. Streptococcus fecalis
   b. Escherichia coli
   c. Proteus mirabilis
   d. Hemophilus influenzae
   [Ref. Harrison 18/e, p 2388]

4. **ELISA test when compared to western blot technique is :** [AI 93]
   a. Less sensitive, less specific
   b. More sensitive, more specific
   c. Less sensitive, more specific
   d. More sensitive, less specific
   [Ref. Park 22/e, p 323]

5. **All are capsulated bacteria except :** [UP 2007]
   a. Neisseria b. Corynebacterium
   c. Hemophillus d. Steptococcus saliverius
   [Ref. Ananthanarayan 8/e, p 232, 9/e, p 236]

6. **Peptidoglycans are present in :** [JIPMER 2000]
   a. Gram '– ve' bacteria b. Gram '+ ve' bacteria
   c. Fungi d. Protozoa
   [Ref. DR Arora 3/e, p 18]

   - Peptidoglycon is present in both Gram +ve (Constitute 50–90% of dry weight of wall) and Gram –ve (constitute 5–10% of dry weight of wall) bacteria.

7. **All are oncogenic viruses except :** [UP 03]
   a. EB virus b. Reo virus
   c. Retrovirus d. Human papilloma virus
   [Ref. Ananthanarayan 8/e, p 563, 9/e 565]

8. **The causative organism of traveller's diarrhea is:**
   a. Shigella b. E. coli [AIIMS 94]
   c. Salmonella d. Viral
   [Ref. Ananthanarayan 8/e 271, 9/e, p 279]

9. **Gram-negative bacteria doesn't take gram stain because it is made of :** [DELHI 08]
   a. Polysaccharide b. Lipopolysaccharide
   c. Techoic acid d. None of the above
   [Ref. Greenwood 18/e, p 14; DR Arora 3/e, p 41]

   - The exact mechanism of gram reaction is not known. The possible reason are:
     - Gram positive bacteria have a more acidic protoplasm, which account for their retaining the basic primary dye more strongly.
     - Dye iodine complex is retained in gram positive cells by thick peptido glycan mesh and is readily wash through the very thin peptidoglycan layer of gram – ve cells.

10. **Which is always present in bacteria ?** [DELHI 2007, 2008]
    a. Cell wall b. Cytoplasmic membrane
    c. Mitochondria d. Nucleoli
    [Ref. Ananthanarayan 8/e, p 13, 19, 9/e, p 16]

11. **Which of the following does not posses both DNA and RNA :** [MP 2001]
    a. Bacteria b. Fungus
    c. Virus d. Spirochete
    [Ref. Ananthanarayan 8/e, p 438, 9/e, p 427]

    - RNA containing virus are called ribovirus.
    - DNA containing virus are called deoxyribovirus.

12. **Most commonly found organism in gut flora :** [Delhi 2007, 2008]
    a. Enterococci b. Bacteroides
    c. E. coli d. Lactobacillus
    [Ref. Jawetz 25/e, p 171]

13. **Anaerobic bacteria surgically most important :** [RJ 2006]
    a. Bacteroides b. Staph aureus
    c. Pseudomonas d. Pneumococcus
    [Ref. Ananthanarayan 8/e, 267, 9/e, p 269]

14. **Acid fast structure is :** [DELHI 2006]
    a. Vibrio b. Nocardia
    c. E. coli d. Bacillus anthracis
    [Ref. Ananthanarayan 8/e, p 392, 9/e, p 393]

    - Nocardia astroides and N. brasiliensis are acid fast.

Answers 1. c. Influenza ... 2. b. Parvo virus 3. d. Hemophilus ... 4. d. More sensitive... 5. b. Coryne...
6. a., b. 7. b. Reo virus 8. b. E. coli 9. d. None... 10. b. Cytoplasmic ...
11. c. Virus 12. b. Bacteroides 13. a. Bacteroides 14. b. Nocardia

15. Infection not transmitted transplacetally is: [DNB 05]
a. Herpes b. EB virus
c. CMV d. Polio
[Ref. Dutta 6/e, p 299]

16. In a splenectomized patient, there is increase of infection by all the organism except : [SGPGI 05]
a. Pneumococci b. Klebsiella
c. H. influenzae d. Neisseria
[Ref. Harrison 18/e, p 471

17. Nocardia resemble actinomyces morphological but : [ICS 2K]
a. Are anaerobic
b. Are faculative anaerobic
c. Are aerobic
d. Require Co2 for growth
[Ref. Ananthanarayan 8/e, p 392, 9/e, p 393]

18. Polysaccharide capsule resistance to phagocytosis is seen in : [Nimhans 01]
a. N. meningitis
b. K. pneumonia
c. Streptococcus pneumonia
d. Yeast
[Ref. Ananthanarayan 8/e, p 221, 9/e, p 223]

19. Which of the following is not cultured : [UP 07, 05]
a. Sporothrix schenckii
b. Rhinosporidium seeberi
c. Candida albicans
d. Aspergillus fumigatus
[Ref. Ananthanarayan 8/e, p 610, 9/e, p 603]

20. The important organism causing meningitis in immunocompromised patient is: [AI 91; Jipmer 91]
a. Histoplasma
b. Cryptococcosis
c. Coccidiomycosis
d. Candida albicans *[Ref. Harrison 18/e, p 1649, 3426]*

21. Most probable cause of food poisoning in a child who has eaten Ice Cream 16-18 hrs. earlier is : [AIIMS 92]
a. Staph aureus
b. Clostridium perfringens
c. Clostridium botulinum
d. Salmonella typhimurium
[Ref. Ananthanarayan 8/e, 299, 9/e, p 300]

22. Which is zoonosis : [RJ 2006]
a. Brucellosis
b. Solmenollosis
c. Shiegellosis
d. Hepatitis *[Ref. Park 22/e, p 732]*

23. Bacteria are motile due to : [RJ 2005]
a. Flagella b. Fimbria
c. Both d. None
[Ref. Ananthanarayan 8/e, p 20, 21, 9/e, p 19, 20]

- Fimbria are not related to motility

24. Which is a live attenuated vaccine : [PGI 93]
a. Rabies b. BCG
c. Hepatitis B d. Cholera
[Ref. Park 22/e, p 98]

25. Which virus does not cause diarrhea : [RJ 2004]
a. Reo virus b. Rota V
c. Adeno virus d. Pox virus
[Ref. Ananthanarayan 8/e, p 463]

26. Fungal infection is diagnosed with : [UP 00]
a. Giemastain b. KOH
c. Foot and pad culture d. Albert strain
[Ref. Ananthanarayan 8/e, 605, 9/e, p 592]

27. The fungi which do not have sexual reproduction belong to which of the following groups : [Kerala 00]
a. Phycomycetes b. Fungi imperfecti
c. Basidomycetes d. Asomycetes
e. None of the above
[Ref. Ananthanarayan 8/e, 601, 9/e, p 590]

28. Teichoic acids : [Kar 00]
a. Are found in the walls of many gram-positive bacteria
b. Make up the outer wall of bacteria
c. Provide receptors for phages
d. Influence the permeability of membrane
[Ref. Jawetz 24/e, p 22]

29. Which of the following is a non culturable fungus: [MAHE 01; UP 05]
a. Rhinosporidium b. Candida
c. Sporothrix d. Penicillium
[Ref. Ananthanarayan 8/e, 610, 9/e, p 603]

30. All the true about bacterial nucleus except : [Kolkata 02]
a. No nuclear membrane
b. Divides by binary fission
c. The bacterial chromosome is diploid
d. No single molecule of double stranded DNA
[Ref. Ananthanarayan 8/e, 19, 9/e, p 18]

31. Louis pasteur is assoicated with : [Kolkata 02]
a. Discovery of the bacillus of tuberculosis
b. The cellular concept of immunity
c. Introduction of anthrax vaccine
d. Discovery of penicillin
[Ref. Ananthanarayan 8/e, p 4, 9/e, p 3]

Answers **15.** d. Polio **16.** b. Klebsiella **17.** c. Are aerobic **18.** c. Streptococcus ... **19.** b. Rhinospori
20. b. Cryptoco... **21.** d. Salmonella **22.** a. Brucellosis **23.** a. Flagella **24.** b. BCG
25. d. Pox virus **26.** b. KOH **27.** b. Fungi ... **28.** a. Are found ... **29.** a. Rhino...
30. c. The bacterial ... **31.** c. Introduction ...

32. Nanometer equals : [Kolkata 02]
a. 10^{-5} mm b. 10^{-3} mm
c. 10^{-4} mm d. 10^{-6} mm

33. The role of plasmids in conjugation was first described by Lederberg and Tatum in : [Kar. 02]
a. Salmonella b. Staph aureus
c. V. cholerae d. Shigella dysenteriae
[Ref. Ananthanarayan 8/e, p 65]

34. The following microorganisms constitutes the normal flora of the oral cavity : [Kar 2002]
a. E. coli
b. Staphylococcus epidermidis
c. Branhamella caterrhalis
d. Picorna virus *[Ref. Jawetz 25/e, p 167]*

35. Obligatory anaerobes cannot withstand oxygen because of absence of : [AP 2005]
a. Superoxide dismutase
b. Catalase *[Ref. Ananthanarayan 9/e, p 24]*
c. Peroxidase
d. Cytochrome oxidase

36. All are transmitted by blood transfusion except :
a. HIV b. Human Parvo virus B 19
c. CMV d. None [AP 04]
[Ref. Harrison 18/e, p 956]

37. Which of the following is not a killed vaccine ?
a. Yellow fever b. Salk (Polio) [Kar 03]
c. Hepatitis B
d. Human diploid cell rabies vaccine
[Ref. Park 22/e, p 99]

38. Which of the following is not an obligate parasite?
a. Virus b. Mycoplasma [Bihar 03]
c. Chlamydia d. Rickettsia
[Ref. Ananthanarayan 8/e, p 386, 9/e, p 386]

39. Mesosomes are : [Bihar 03]
a. Respiratory enzymes in bacteria
b. Cytoplasmic invagination
c. Destructive bodies
d. Protein forming bodies
[Ref. Ananthanarayan 8/e, p 18, 9/e, p 17]

40. The following are motile organisms except : [DNB 04]
a. Proteus b. Diphtherias
c. Clostridia d. Anthrax
[Ref. Ananthanarayan 8/e, p 232, 9/e, p 236]

41. The biochemial composition of bacterial endotoxin is :
a. Peptidoglycan b. Lipopolysaccharide
c. Glycopeptids d. Lipoprotein [MP 05]
[Ref. Ananthanarayan 8/e, p 79, 9/e, p 74]

42. Pigment producing colonies are seen in : [Kar 04]
a. Pseudomonas b. Atypical mycobacteria
c. Serratia marcescens d. All of the above
[Ref. Ananthanarayan 7/e, p 366, 282, 319]

43. Positive sense, RNA genome are found in : [UP 04; Kerala 01]
a. Poliovirus b. Papova virus
c. Influenza virus d. Picorna virus
[Ref. Ananthanarayan 8/e, p 430, 9/e, p 484, 433]

44. Brain abscess in immunodeficient person is due to :
a. Cryptococus b. Staphylococcus
c. Pneumococcus d. E. coli [Jharkhand 04]
[Ref. Harrison 18/e,p 3428]

Etiology of Brain Abscess

Immunocompetant	Immunodeficient
• Streptococcus	• Nocardia
• Proteus	• Toxoplasma gondii
• E. coli	• Aspergillus
• Klebsiella	• Candida
• Bacteroides	• C. neoformans
• Fusobacterium	
• Staphylococci	

In India most common cause of brain abscess is M. tuberculosis

45. Bacterial cell wall is composed of all except : [Bihar 04]
a. Muramic acid b. Teichoic acid
c. Glucosamine d. Mucopeptide
[Ref. Ananthanarayan 8/e, p 17, 9/e, p 16]

46. Bacterial capsule is made up of : [Bihar 04]
a. Monosaccharide b. Polysaccharide
c. Long chain fatty acid d. Small chain fatty acid
[Ref. Jawetz 24/e, p 31]

47. Condyloma accuminatum caused by human papilloma virus (HPV) types of : [UP 05]
a. 18, 31 b. 17, 12
c. 6, 11 d. 16, 18
[Ref. Ananthanarayan 9/e, p 553]

48. Virus growth in cell cultures detected by cytopathic effect characteristic features of : [UP 05]
a. Syncytium formation b. Budding formation
c. Flattening d. All of the above
[Ref. Ananthanarayan 8/e, p 435, 9/e, p 436]

49. Which among is not a fungus : [Jharkhand 05]
a. Rhinosporidiosis b. Sporrotrichosis
c. Torulois d. Candidiasis
[Ref. Chakarborty 2/e, p 610]

Answers **32.** d. 10–6 mm **33.** d. Shigella ... **34.** c. Branhamella ... **35.** b. Catalase... **36.** d. None
37. a. Yellow fever **38.** b. Mycoplasma **39.** a. Respiratory ... **40.** b. Diphtherias **41.** b. Lipopolysac ...
42. d. All of the ... **43.** d. Picorna virus **44.** a. Cryptococus **45.** b. Teichoic acid **46.** b. Polysac ...
47. c. 6, 11 **48.** a. Syncytium ... **49.** c. Torulois

50. Mesosomes in bacteria are functional unit for :
a. Lipid storage [Bihar 05]
b. Protein synthesis
c. Respiratory enzymes
d. None
[Ref. Ananthanarayan 8/e, p 18, 9/e, p 17]

51. Most common organism causing lobar pneumonia:
a. Klebsiella pneumoniae [MP 06]
b. Streptococci pneumoniae
c. H. influenzae
d. Proteus
[Ref. Harrison 18/e, p 2131]

52. Most common organism in gut is : [MP 06]
a. E. coli b. Lactobacillus
c. Bacteroides d. Klebsiella
[Ref. Jawetz 25/e, p 171]

53. Peptidoglycans are present in : [UP 2006]
a. Gram –ve bacteria b. Gram +ve bacteria
c. Both a and b d. Protozoa
[Ref. DR Arora 3/e, p 18]

54. Cell wall structure is found in all except : [UP 06]
a. Staph aureus
b. Pseudomonas aeroginosa
c. Mycoplasma pleumoniae
d. Corynebacterium diphtheriae
[Ref. Ananthanarayan 8/e, p 387, 9/e, p 14]

55. Vertically transmitted disease caused by all except: [UP 06]
a. Toxoplsma
b. Cytomegala
c. HIV
d. Treponema pertenue *[Ref. Park 22/e, p 93]*

56. All the disease caused by transfusion except : [UP 06]
a. HIV
b. Hepatitis-B
c. Hepatitis-C
d. Plague *[Ref. Harrison 18/e, p 956]*

57. The cytoplasmic membrane bacteria is responsible for: [Kar 06]
a. Selective permeability
b. Motility
c. Cell division
d. Conjugation *[Ref. Ananthanarayan 9/e, p 16]*

58. In negative staining : [Kar 06]
a. The structure to be demonstrated is stained
b. The structure to be demonstrated is not stained
c. The background is not stained
d. The background and structure are stained
[Ref. Ananthanarayan 8/e, p 14, 9/e, p 12]

59. Not used in Gram's staining. [DNB 2012, 09]
a Methylene blue
b Crystal violet
c Iodine
d Safranin
[Ref. Greenwood 18/e 14]

60. Louis Pasteur was associated with all EXCEPT:
a Vaccination of small pox [DNB 2007]
b Germ theory
c Pasteurization
d Vaccination of rabies

Small pox vaccine was invented by E. Jenner

61. Stain use for staining degenerated fungi in tissue is:
a. PAS [DNB 2009]
b. Gomori methamine silver
c. H and E
d. Muciramine *[Ref: Greenwood 18/e 619]*

Gomori-Methenamine silver (GMS) and periodic acid-schiff (PAS) are the two most common stains used to look for fungi in tissues and in cytology specimens

62. Antonie van Leeuwenhoeck is associated with?
a. Telescope [DNB 2012]
b. Microscope
c. Stains
d. Immunization
[Ref. Ananthanarayan 9/e, p 3]

63. "Yellowblack" granules are seen in which fungal infection. [DNB 2012]
a. Mucormycosis
b. Mycetoma
c. Aspergillosis
d. Rhinosporidiosis
[Ref. Ananthanarayan 8/e p 608, 9/e, p 601]

64. Phenol test or Reidel walker test is done to determine: [DNB 2012]
a. Hardness of water
b. Chlorine demand
c. Quality of disinfectant
d. Efficacy of a disinfectants
[Ref. Ananthanarayan 9/e 36]

Answers **50.** c. Respiratory... **51.** b. Streptococci ... **52.** c. Bacteroides **53.** b. Gram +ve ... **54.** c. Mycoplas ...
55. d. Treponema ... **56.** d. Plague **57.** a. Selective ... **58.** b. The structure ...
59. a. Methylene blue **60.** a. Vaccination ... **61.** a. PAS; b. Gomori.. **62.** b. Microscope **63.** b. Mycetoma
64. d. Efficacy of a disinfectants

Reid walker test is done to determine the efficacy of a disinfectant in the terms of phenol. The dilution of disinfectant which sterilizes the suspension in a given time, divided by the corresponding dilution of phenol and is stated as phenol coefficient of the said disinfectant.

65. All oncogenic viruses containing RNA belongs to:
a. Picronaviridae **[DNB 2013]**
b. Herpesviridae
c. Retroviridae
d. Orthomyo virus *[Ref. Ananthanarayan 9/e, p 566]*

66. Fugal staining is done by:
a. Calcoflor white **[DNB 2013]**
b. Leishman stain
c. Z-N staining
d. None *[Ref. Ananthanarayan 9/e, p 592]*

67. True about anaerobic infection:
a. Causes toxemia **[DNB 2013]**
b. Cause systemic infection
c. Both
d. None *[Ref. Ananthanarayan 9/e, p 251]*

68. Spores are formed by all except:
a. E. coli **[DNB 2013]**
b. B. anthrex
c. B. cerus
d. None *[Ref. Ananthanarayan 9/e, p 272]*

69. Capsulated fungi is:
a. Cryptococcus **[DNB 2013]**
b. Rhinosporidium
c. Both
d. None *[Ref. Ananthanarayan 9/e, p 613]*

Answers **65.** c. Retroviridae **66.** a. Calcoflor white **67.** c. Both **68.** a. E. coli **69.** a. Cryptococcus

NEET Pattern Questions

1. **Eukaryoes are different in causing infection because:**
   a. Diyide by biniary fission
   b. Highly structured cell with organized cell organelles
   c. Donot have all organelles
   d. Evolutionally ancient *[Ref: Ananthanarayan 9/e p 10]*

2. **Ehrlichia chaffeensis is causative agents of:**
   a. HME
   b. HGE
   c. Glandular fever
   d. None *[Ref: Ananthanarayan 9/e p 409]*

- HME refers to human monocytic ehrlichiosis caused by Ehrlichia chaffeensis. It is transmitted by Amblyomma ticks. Deer and rodents are the reservoir hosts.

Ehrlichia

- Small, Gram negative obligate intracellular bacteria which have an affinities towards blood cells.
- They grow within phagosomes as mulberry like clusters and are transmitted by ticks.

Pathogenicity

- Human Monocytic Ehrlichiosis: Caused by E. chaffeenisis.
- Human granulocytic Ehrlichiosis: Causes by E. equi.
  - In both conditions there is thrombocytopenia and leukopenis.
- Glandular Fever: Caused by Ehrlichia sennetsu
  - There is lymphoid hyprerplasia with atypical lymphocytosis.
- Doxycline is the treatments of choice.

3. **Correct order of gram staining is:** *[Ref: Ananthanarayan 9/e p 13]*
   a. Gention violet → Iodine Carbol→ fuchsin
   b. Iodine → Gention violet → Carbol fuchsin
   c. Carbol fuchsin → Iodine → Gention violet
   d. Carbol fuchsin → Gention violet → iodine

Gram's staining procedure.

- Primary staining with pararosaniline dye such as crystal violet methyl violet and gentian violet.
- Application of dilute solution of iodine.
- Decolourisation with an organic solvents such as ethanol, acetone or aniline.
- Counter staining with a dye of contrasting colour such as carbol fuchsin safranine or neutral red.

4. **Not a component of Gram stain:** *[Ref. Ananthanarayan 9/e, p 13]*
   a. Methylene blue b. Ethanol
   c. Iodine d. Gentian violet

5. **Which part of bacteria is most antigenic:** *[Ref. Ananthanarayan 9/e, p 88]*
   a. Protein coat b. Lipopolysaccharide
   c. Nucleic acid d. Lipids

6. **Which of the following is a protista:**
   a. Algae b. Fungi
   c. Proteus d. Bacteria

7. **Non-motile organism:** *[Ref. Ananthanarayan 9/e, p 285]*
   a. E. coli b. Vibrio cholera
   c. Protein in nature d. Antigenic

Motile bacteria

Polar Flagella,	Peri-trichous flagella
• Vibrio	• E.coli
• Pseudomonas	• Proteus
• H. pylori	• Listeria monocytogenes
• Campylobactor	• All clostridia except Cl. perfringens and and Cl. tetani VI
• Spirochetes	• Bacillus except B. anthrax
• Legionella	• Salmonella except S. gallinarum-pullorum

8. **Flagella not true:** *[Ref. Ananthanarayan 9/e, p 19]*
   a. Locomotion
   b. Attachment
   c. Protein in nature
   d. Antigenic

Flagella is made up of protein called by flagellin, similar to keratin or myosin

- Because of their proteinecious nature they are antigenic.

9. **Which of the following organisms does not enters through abrasions in the skin:**
   a. E. rhusiopathiae
   b. E. corrodens
   c. C. hominis
   d. C. violaceum

Answers 1. b. Highly.... 2. a. HME 3. a. Gention violet... 4. a. Methylene blue
5. a. Protein coat 6. c. Proteus 7. d. Antigenic 8. b. Attachment
9. c. C. hominis

Organisms entering the body through breaks in the skin or mucous membranes.

Aerobic and facultative microorganisms	Anaerobic bacteria	Aerobic microorganism from unusual, specialized and zonotif infections	Yeast
• Coagulase negative staphylococci • Staphylococcus aureus • Enterococcus spp. • Streptococcus viridans • Coryncbacterium spp. • Bacillus cereus • E.coli • Serratia • Enterobactor • Proteus • Morganella • Pseudomona • Acinetobactor	• Peptostreptococcus spp. • Clostridium spp. • Ebacterium limposum • Bacteriodes fragilis • Prevotella spp. • Prophyromonas • Fusobacterium • Villonella spp.	• Actinobacillus actinomyceteracomitans • Aeromonas spp. • Bacillus anthracis • Bergeyella zoohelcum • Chromobacterium violaceum • Eikenella corrodens • Erysipalothrix rhusiopathial • Francisella tularensis • Haemophillus spp. • Kingella kingae • Pasteurella multocida • Streptobacillus moniliformis • Vibro vulnificus	• Candida albicans • Candida krusei • Candida parapsilosis

10. True about exotoxin:
a. Non-antigenic
b. Enzymatic
c. Non-protein
d. Heat stable
[Ref. Ananthanarayan 9/e, p 75]

11. Smallest virus is:
a. Herpes virus
b. Adenovirus
c. Parvovirus
d. Poxviurs
[Ref. Ananthanarayan 9/e, p 554]

12. KOH wet mount is prepared for:
a. Herpes Zoster
b. Candida
c. Gonorrhea
d. Trichomonas vaginalis
[Ref. Ananthanarayan 9/e, p 592]

KOH wet amount digest cell and other tissue material and thus aid in better visualization of fungi

13. Thermophile bacteria grow at:
a. 20°C
b. 20–40°C
c. 20–60°C
d. 60–80°C
[Ref. Ananthanarayan 9/e, p 25]

Bacteria

Mesopholic	**Psychrophilic**	**Thermophiles**
grows best at room temperature 25-40°C	grows best at temperature <20°C	grows best at 55-80°C eg. B-stearothermohilus

Note: Extremely thermophilic bacterias have been identified which can grow at temperature as high as 250°C

14. Healthy carriers are seen in all except:
a. Polio
b. Meningococci
c. Measles
d. Cholera
[Ref. Park 22/e, p 92]

15. Rash is not caused by:
a. Salmonella
b. Shigella
c. Meningococci
d. Staphylococcus
[Ref. Harrison 19/e, p 128]

16. Which vaccine can cause adverse effects in persons with allergy to egg:
a. Measles
b. Rubella
c. Rabies
d. Mumps

Vaccines that contain small quantities of egg protein can cause hypersensitivity reactions in some peoples with allergies to egg Eg.
- MMR vaccine
- MMRV vaccine
- Influenzae vaccine
- Yellow fever vaccine
- RabAvert rabies vaccine
- Yellow fever vaccine

Answers 10. b. Enzymatic 11. c. Parvovirus 12. b. Candida 13. d. 60–80°C
14. c. Measles 15. b. Shigella 16. c. Rabies

17. **Pediculus humanus can transmitt:**
    a. Sleeping sickness
    b. Plague
    c. Chaga's disease
    d. Relapsing fever *[Ref. Park 22/e, p 712]*

18. **Southern blotting is used for:**
    a. RNA
    b. DNA
    c. Protein
    d. Antibody

> **Southern blot:** Used for defecting specific DNA sequence in DNA samples.
> **Western blot:** Used to detect specific protein
> **Northern blot:** Used to detect specific RNA
> **Eastern blot:** Used to analyze protein post translational modifications such as lipids.
> **Southwestern blot:** Used to identifying and characterizing DNA binding proteins.

19. **Rhinosporidiosis is caused by:**
    a. Fungus
    b. Protozoa
    c. Aquatic bacterium
    d. Protista

20. **Disease transmitted from men to animals:**
    a. Antropozoonoses
    b. Zooanthroponoses
    c. Amphixenoses
    d. Aptozoonoses *[Ref. Park 22/e, p 19]*

> **Anthropozoorosis:** Infection transmitted to man from vertebrate animals eg. rabies.
> **Zooanthroponoses:** Infection transmitted from man to vertebrate animals.
> **Amphisenosis:** Infection maintained in both man and lower vertebrate animals that may be transmitted in either direction e.g. T. Cruzi.

21. **All are true about chromobacterium violaceum except:** *[Ref. Ananthanarayan 9/e, p 397]*
    a. Gram negative
    b. Produces violet-colored pigment
    c. Normal flora in human
    d. Causes cellulitis

> **Chromobacterium violaceum**
> - Gram negative nonsporing bacillus
> - Motile with lateral flagella
> - Facultative anaerobes growing on ordinary media and produce violet pigment
> - Human infections are recorded in tropics and consist of multiple abscess, cellulites, pyemia.

22. **Capsid of viral structure is:**
    a. Extracellular infectious particle
    b. Protein coat around nucleic acid
    c. Envelop around a virus
    d. None of the above *[Ref. Ananthanarayan 9/e, p 428]*

> - Protein coat surrounding virion is called capsid
> - Capsid with the enclosed nucleic acid is known as nucleocapsid.

Answers 17. c. Chaga's disease 18. b. DNA 19. a. Fungus 20. b. Zooanthroponoses
21. c. Normal flora in human 22. b. Protein coat around nucleic acid

Section - C

Emerging Diseases

- Swine Flu
- Zika Virus

CHAPTER

40

Swine Flu

Swine influenza (also called **Pig influenza, swine flu, hog flu and pig flu)** is an infection by any one of several types of swine influenza virus. **Swine influenza virus (SIV) or S-OIV (swine-origin influenza virus)** is any strain of the influenza family of viruses that is endemic in pigs. The known SIV strains include influenza C and the subtypes of influenza A known as **H1N1**, H1N2, H3N1, H3N2, and H2N3.

The **2009 flu pandemic** is a global outbreak of a new strain of H1N1 influenza virus, It was first detected in April 2009, contains a **combination of genes** from swine, avian (bird), and human influenza viruses.

The outbreak began in Veracruz, Mexico. The pandemic virus is a type of swine influenza, derived originally from a strain that lived in pigs and this origin gave rise to the common name of "swine flu". However, despite its origin in pigs, this strain is transmitted between people and not from swine to people. The United States Secretary of Agriculture made clear that despite its common name being "swine flu", there is no risk of contracting flu from eating cooked pork products.

TRANSMISSION

Transmission between pigs

- Influenza is common in pigs, about half of breeding pigs have been exposed to influenza.
- The main route of transmission is through direct contact between infected and uninfected animals. Airborne transmission through the aerosols produced by pigs coughing or sneezing are also an important means of infection.
- However, pigs are unusual as they can be infected with influenza strains of different species: pigs, birds and humans. *This makes pigs a host where influenza viruses might exchange genes, producing new and dangerous strains.*

Transmission to humans

- People who work with poultry and swine, especially people with intense exposures, are at increased risk of zoonotic infection with influenza virus
- Other professions at particular risk of infection are veterinarians and meat processing workers, although the risk of infection for both of these groups is lower than that of farm workers.

Transmission in 2009 pandemic

- In human H1N1 virus spread occur in the same way that seasonal flu spreads. Flu viruses are spread mainly from person to person through coughing or sneezing by people with H1N1 infection.
- Sometimes people may become infected by touching something – such as a surface or object – with flu viruses on it and then touching their mouth or nose.
- ***The basic reproduction number*** (the average number of other individuals that each infected individual will infect, in a population that has no immunity to the disease) for the 2009 novel H1N1 is estimated to be 1.75

Signs and symptoms

The symptoms of swine flu are similar to other influenzas, and include fever, cough *(typically a "dry cough")*, headache, muscle or joint pain, sore throat, chills, fatigue, and runny nose. Diarrhea, vomiting, and neurological problems have also been reported in some cases.

People at higher risk of serious complications include:

- Age over 65, children younger than 5,
- Children with neurodevelopmental conditions,
- Pregnant women (especially during the third trimester),
- Underlying medical conditions, such as asthma, diabetes, obesity, heart disease, or a weakened immune system.

Symptoms in severe cases

The World Health Organization reports that the clinical picture in severe cases is strikingly different from the disease pattern In severe cases, patients generally begin to deteriorate around 3 to 5 days after symptom onset. Deterioration is rapid, with many patients progressing to respiratory failure within 24 hours.

A November 2009 CDC recommendation stated that the following constitute "emergency warning signs" which demands urgent hospitalization include:

In adults:

- Difficulty breathing or shortness of breath
- Pain or pressure in the chest or abdomen
- Sudden dizziness
- Confusion
- Severe or persistent vomiting
- Low temperature

In children:

- Fast breathing or working hard to breathe
- Bluish skin color
- Not drinking enough fluids
- Not waking up or not interacting
- Being so irritable that the child does not want to be held
- Flu-like symptoms that improve but then return with fever and worse cough
- Fever with a rash
- Being unable to eat
- Having no tears when crying

Diagnosis

Confirmed diagnosis of pandemic H1N1/09 flu requires testing of a nasopharyngeal, nasal, or oropharyngeal tissue swab from the patient. Real-time RT-PCR is the recommended test as others are unable to differentiate between pandemic H1N1/09 and regular seasonal flu.

Treatment

- A number of methods have been recommended to help ease symptoms, including adequate liquid intake and rest.
- Over-the-counters pain medications such as acetaminophen and ibuprofen do not kill the virus, but reduce symptoms. Aspirin and other salicylate products should not be used with any flu-type symptoms because of the risk of developing Reye's Syndrome.
- If the fever is mild and there are no other complications, fever medication is not recommended.
- People in at-risk groups should be treated with antivirals (oseltamivir or zanamivir) as soon as possible when they first experience flu symptoms. Antivirals are most useful if given within 48 hours of the start of symptoms and may improve outcomes in hospitalized patients
- Normal individuals who have persistent or rapidly worsening symptoms should also be treated with antivirals.
- If oseltamivir (Tamiflu) is unavailable or cannot be used zanamivir (Relenza) is recommended as a substitute. Peramivir is another antiviral drug approved for hospitalized patients in cases where the other drugs are ineffective or unavailable.

PREVENTION

Vaccine

Two types of influenza vaccines are available:

- TIV [Flu shot (injection) of trivalent (three strains; usually A/H1N1, A/H3N2, and B) inactivated (killed) vaccine] or
- LAIV [nasal spray (mist) of live attenuated influenza vaccine.]
  - LAIV is not recommended for individuals under age 2 or over age 50, but might be comparatively more effective among children over age 2.
  - Children through 9 years of age should get two doses of vaccine, about a month apart. Older children and adults need only one dose.

CHAPTER

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Zika Virus

Zika virus is an emerging mosquito-borne virus that was first identified in Uganda in 1947 in rhesus monkeys through a monitoring network of sylvatic yellow fever. It was subsequently identified in humans in 1952 in Uganda and the United Republic of Tanzania. Outbreaks of Zika virus disease have been recorded in Africa, the Americas, Asia and the Pacific

Etiology:

- Zika virus is single stranded RNA virus belongs to family flavii virus.
- **Vector**: Aedes mosquitoes (which usually bite during the morning and late afternoon/evening hours)
- **Reservoir:** Unknown

Signs and Symptoms:

- The incubation period (not known exactly), but is likely to be a few days.
- The symptoms are similar to other arbovirus infections such as dengue, and include fever, skin rashes, conjunctivitis, muscle and joint pain, malaise, and headache. These symptoms are usually mild and last for 2-7 days.
- Enhances the risk of Guillain-Barré syndrome
- There are evidences that Zika virus enhances the chances of microcephaly if infection is acquired in pregnancy

Transmission:

- Zika virus is transmitted to people through the bite of an infected mosquito from theAedes genus, mainly Aedes aegypti in tropical regions.
- Sexual transmission of Zika virus has also been described.

Diagnosis:

- Infection with Zika virus may be suspected based on symptoms and recent history (e.g. residence or travel to an area where Zika virus is known to be present).
- Zika virus diagnosis can only be confirmed by laboratory testing for the presence of Zika virus RNA in the blood or other body fluids, such as urine or saliva.

Treatment:

- Zika virus disease is usually relatively mild and requires no specific treatment.
- People sick with Zika virus should get plenty of rest, drink enough fluids, and treat pain and fever with common medicines.
- If symptoms worsen, they should seek medical care and advice. There is currently no vaccine available.

Prevention:

- As disease is transmitted through mosquito bite, prevention and control relies on reducing mosquitoes.
- This can be done by using insect repellent regularly; wearing clothes (preferably light-coloured) that cover as much of the body as possible; using physical barriers such as window screens, closed doors and windows;
- People (particularly pregnant females) should avoid travelling in areas with out break or if at all necessary travellers should take the basic precautions described above to protect themselves from mosquito bites.

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